Exploring the boundaries of precision haemato-oncology

The case of FLT3 length mutated acute myeloid leukaemia

Caroline Benedicte Nitter Engen

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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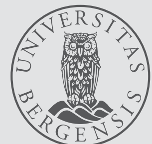
UNIVERSITY OF BERGEN



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“To wrest from nature the secrets which have perplexed philosophers in all ages, to track to their sources the causes of disease, to correlate the vast stores of knowledge, that they may be quickly available for the prevention and cure of disease – these are our ambitions.”

Sir William Osler, 1902

Scientific environment

This thesis was initiated in September 2013 and completed November 2019. The work has mainly been performed within the "Precision Oncology Research Group", headed by Professor Bjørn Tore Gjertsen. The group is organised under the Institute for Clinical Science (K2) at the Faculty of Medicine and Odontology (MOFA) at the University of Bergen (UiB). The work has been supervised by Professor Bjørn Tore Gjertsen, in collaboration with co-supervisors Professor Emmet McCormack and Professor Øystein Bruserud.

Part of the work has been performed in close collaboration with other academic groups, both locally in Bergen as well as at sites of national and international partners. At the Department of Medical Genetics at Haukeland University Hospital Randi Hovland and Atle Brendehaug has contributed with experience, technical support and data analysis with regards to conventional genetic analysis like karyotyping, fluorescence in situ hybridisation, polymerase chain reaction and Sanger sequencing. Internationally the work has been performed in collaboration with the Finnish Institute for Molecular Biology (FIMM), primarily through principal investigator Caroline Heckman and her group. They have contributed with drug sensitivity and resistance testing as well as next generation sequencing experiments. The work is also a result of a collaboration with the HOVON consortium, principally through Peter Valk and Bob Löwenberg. They have provided clinical samples and clinical data. Jonathan Irish and Brent Ferrell from Vanderbilt University in Nashville have contributed with mass cytometry experiments.

A PhD degree is not merely the sum of the thesis and the manuscripts, but comprise a professional progress embedded in a larger cultural context. The scientific environments contributing to this includes The Centre for Cancer Biomarkers (CCBIO) and The Centre for the Study of the Sciences and the Humanities (SVT) at the University of Bergen (UiB).

The PhD Fellowship was granted by the University of Bergen (UiB). Additional financial contributors include the Norwegian Cancer Society, the Western Norway Health Authority (Helse Bergen), The Norwegian Research Council, and the Meltzer fond. Celgene has provided travel grants.

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I want to express my deepest gratitude to Roger Strand, for his continuous inspiration, introducing me to the theory and philosophy of science, as well as science and technology studies; expanding my literacy, and making my intellectual journey deeper and richer, both literally and metaphorically. I also owe great gratitude to Monica Hellesøy and Calum Leitch, my partners in heterodox positions as founders of "Thoughts On Precision". Our discussions and deliberations on cancer and cancer research are inseparable from this thesis work. I further want to thank "Filosofisk Poliklinikk", inviting me to be part of their board, and together challenging and expanding the traditional borders of medicine.

From the depth of my heart I want to express my gratitude to my family. I would not be here today without the lifelong support and love of my parents Astrid and Sverre, always stimulating me and inspiring me to pursue my ambitions and goals. At the core of my world my husband Andrea and my children Matteo and Lea – Thank you for providing love and purpose to my life - which essentially is all that matters!

Bergen, November 2019

Caroline Benedicte Nitter Engen

Abbreviations

AKT	AKT Serine/Threonine Kinase
AML	Acute myeloid leukaemia
ASXL1	Additional Sex Combs Like 1, Transcriptional Regulator
ATP	Adenosine triphosphate
ATRX	Alpha Thalassemia/Mental Retardation Syndrome X-Linked
BCR-ABL	Breakpoint Cluster Region - Abelson murine leukemia viral oncogene homolog 1
BRAF	B-Raf Proto-Oncogene, Serine/Threonine Kinase
BTG1	B-Cell Translocation Gene 1
CBL	Casitas B-Lineage Lymphoma Proto-Oncogene
CCL3	C-C Motif Chemokine Ligand 3
CD	Cluster of Differentiation
CIC	Capicua Transcriptional Repressor
cKIT	KIT Proto-Oncogene,
CSF1R	Colony Stimulating Factor 1 Receptor
CTNNB1	Catenin Beta 1
CyTOF	Cytometry by Time of Flight
DNA	Deoxyribonucleic acid
EGFR	Epidermal Growth Factor Receptor
ELN	European LeukemiaNet
ERK	Extracellular signal–regulated kinases
FGFR2	Fibroblast Growth Factor Receptor 2
FLT3	Fms-Like Tyrosine Kinase 3
FUBP1	Far Upstream Element Binding Protein 1
GM-CSF	Granulocyte-macrophage colony-stimulating factor
IDH1	Isocitrate Dehydrogenase 1
IDH2	Isocitrate Dehydrogenase 2
IL-3	Interleukin 3
IL2RG	Interleukin 2 Receptor Subunit Gamma
ITD	Internal tandem duplication
JAK	Janus Kinase
KRAS	Kirsten Rat Sarcoma Viral Proto-Oncogene
LM	Length mutation
MAPK	Mitogen-Activated Protein Kinase
MEK	Mitogen-activated protein kinase
MLL	Mixed-Lineage Leukemia
mTOR	Mechanistic Target Of Rapamycin Kinase
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
NOD/SCID	Nonobese diabetic/severe combined immunodeficiency
NOTCH1	Notch Receptor 1
NPM1	Nucleophosmin 1
NRAS	neuroblastoma RAS viral oncogene homolog

NSG	Nonobese diabetic/severe combined immunodeficiency gamma
NSGS	Nonobese diabetic/severe combined immunodeficiency gamma-SGM3
PCR	Polymerase Chain Reaction
PDGFR	Platelet Derived Growth Factor Receptor
PI3K	Phosphatidylinositol 3-kinases
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
PMC	PubMed Central
PMID	PubMed Unique Identifier
PML-RARA	Promyelocytic Leukemia - Retinoic Acid Receptor Alpha
PTEN	Phosphatase And Tensin Homolog
PTPN11	Protein Tyrosine Phosphatase Non-Receptor Type 11
RAF	RAF Proto-Oncogene Serine/Threonine-Protein Kinase
RAS	RAS Proto-Oncogene
RNA	Ribonucleic acid
RUNX1	Runt-related transcription factor 1
RUNX1T1	RUNX1 Partner Transcriptional Co-Repressor 1
SCF	Stem cell factor
STAT	Signal Transducer and Activator Of Transcription
TET2	Ten-Eleven Translocation Methylcytosine Dioxygenase 2
TKD	Tyrosine kinase domain
TP53	Tumour Protein P53
WHO	World Health Organisation

Summary

This dissertation explores the astounding biological heterogeneity of acute myeloid leukaemia (AML). In three research papers, we study *FLT3* length mutated (LM) AML to contribute to the characterisation of genetic diversity between patients but also within the same patient as the disease develops over time.

AML is a rare but severe blood cancer for which outcome is poor. In haemato-oncology as in other cancer fields, the imaginary of precision medicine is a potent force that provides direction to research as well as the development of clinical practice. Specifically, the hope is to tailor cancer management by molecular profiling and therapeutic targeting of actionable aberrations. In the case of AML, the validity and utility of this approach is an open empirical question. The aim of this dissertation is to explore the biological boundaries of precision oncology in the context of AML. I have pursued this aim through empirical characterisation of AML as a heterogeneous and dynamic phenomenon (in the research papers) and a literature study and theoretical reflection on the relationship between that empirical characterisation and available conceptual frameworks of cancer (in the synthesis part of the dissertation).

Somatic mutation theory, tumour evolution theory and cancer stem cell theory were identified as the prevailing conceptual frameworks representing variants of explanatory models in AML. These theories also provide justification for the clinical approaches related to precision haemato-oncology. The synthesis provides an overview of empirical *FLT3*-LM AML research poorly accounted for by these prevailing models, including intra-leukaemic plurality of *FLT3*-LMs as well as clinico-pathological relationships that suggest context-dependency with regards to *FLT3*-LM properties. Indeed, in Paper I we confirm the finding that AML patients may have several *FLT3*-LMs. In Paper II, we show hitherto undiscovered sex differences in genetic profiles of *FLT3*-LM AML. In Paper III, a single patient is followed through the course of his disease to reveal how genetically diverse cell populations may initiate leukaemia in animal models.

These findings pose challenges to the understanding of *FLT3*-LMs as causal contributors in AML pathogenesis. A major conclusion of this dissertation is that somatic mutation theory is not a sufficient conceptual framework for AML. Evolutionary perspectives seem called for, and the dissertation proposes that a selection-centric perspective to further the understanding and interpretation of *FLT3*-LM AML pathogenesis, conceptualising both cancer cells and cancer as dynamic phenomena rather than confined entities. Based on this I propose a shift towards characterisation of permissive conditions facilitating the emergence and persistence of disease.

Sammendrag

Dette doktorgradsarbeidet utforsker biologisk heterogenitet i kreftsykdommen *akutt myeloid leukemi* (AML). I tre forskningsartikler studerer vi *FLT3* lengdemutert (LM) AML og viser at slik heterogenitet fins mellom pasienter, men også innenfor samme pasient etter hvert som sykdommen utvikler seg over tid.

AML er en sjelden, men alvorlig form for blodkreft. Også innenfor forskningen på blodkreft råder det for tida en visjon om *presisjonsmedisin.* Denne visjonen preger både forskning så vel som klinisk praksis. Håpet er å kunne skreddersy kreftbehandling ved hjelp av molekylær profilering og målretting behandling. For AML er nytteverdien av denne tilnærmingen fremdeles et åpent spørsmål. Målet med denne avhandlingen er å utforske biologiske forhold i AML som gir begrensninger for presisjonsmedisin. Avhandlingen dokumenterer at AML er et heterogent og dynamisk fenomen. Dette diskuteres opp mot begrepene og modellene som forskningslitteraturen på feltet anvender for å forstå *FLT3*-LM AML.

Avhandlingen viser at somatisk mutasjonsteori, tumor-evolusjonsteori og kreftstamcelleteori er de rådende forklaringsmodellene i AML. Disse teoriene benyttes også til å legitimere kliniske tilnærminger i hemato-onkologi. Avhandlingens kappe gir en oversikt over funn i *FLT3*-LM AML-forskningen som vanskelig kan forklares av de nevnte modellene, blant annet intra-leukemisk mangfold av *FLT3*-LMs så vel som klinisk-patologiske forhold som tyder på at egenskapene til *FLT3*-LMs avhenger av konteksten mutasjonene befinner seg i. I artikkel I bekrefter vi funnet at AML-pasienter kan ha flere *FLT3*-LM-er. I artikkel II viser vi hittil uoppdagede kjønnsforskjeller i genetiske profiler av *FLT3*-LM AML. I artikkel III blir en enkelt pasient fulgt gjennom sykdomsforløpet og vi avdekker hvordan genetisk forskjellige cellepopulasjoner fra denne pasienten initierer leukemi i dyremodeller.

Samlet sett utfordrer disse funnene den rådende forståelsen av *FLT3*-LM som årsak til AML. En hovedkonklusjon i avhandlingen er således at somatisk mutasjonsteori ikke gir en adekvat beskrivelse av patogenesen av AML. I stedet framstår evolusjonsteoretiske perspektiver som mer lovende. Avhandlingen foreslår at et seleksjonssentrert perspektiv kan øke forståelsen av hvordan *FLT3*-LM AML oppstår. Både kreftceller og kreft bør forstås som dynamiske fenomener. På bakgrunn av dette foreslår avhandlingen at det gis økt oppmerksomhet mot å karakterisere forhold som tillater at kreftsykdom oppstår, opprettholdes og ekspanderer.

List of manuscripts

PAPER I:

Engen C, Grob T, Hinai A, Hellesøy M, Brendehaug A, Wergeland L, Bedringaas SL, Hovland R, Valk P and Gjertsen BT. **FLT3-ITD mutations in acute myeloid leukaemia – molecular characteristics, distribution and numerical variation.** *Manuscript*

PAPER II

Engen C, Grob T, Hellesøy M, Löwenberg B, Valk P and Gjertsen BT. **Sex and FLT3-ITD mutation status in acute myeloid leukaemia.** *Manuscript*

PAPER III

Engen C, Dowling TH, Hellesøy M, Eldfors S, Ferrel B, Gullaksen SE, Popa M, Brendehaug A, Karjalainen R, Mejlænder-Andersen E, Majumder MM, Kimmo P, Hovland R, Bruserud Ø, Irish J, Heckman C, McCormack E, and Gjertsen BT. **Converging molecular evolution in acute myeloid leukaemia.** *Manuscript*

Table of content

SCIENTIFIC ENVIRONMENT .... **II**
ACKNOWLEDGEMENTS .... **III**
ABBREVIATIONS .... **IV**
SUMMARY .... **VI**
SAMMENDRAG .... **VII**
LIST OF MANUSCRIPTS .... **VIII**
TABLE OF CONTENT .... **IX**
INTRODUCTION **1**
PREFACE 1
PRECISION MEDICINE – TRADITION, EVIDENCE, REASON AND AMBITION 3
PRECISION ONCOLOGY – EXPECTATIONS AND REALISATIONS 5
THE CASE 6
Methodological approach of the synthesis part of the dissertation *8*
Acute myeloid leukaemia *9*
FMS-like tyrosine kinase receptor 3 .... *11*
THEORETICAL FRAMEWORKS IN AML 14
Somatic mutation theory *16*
Tumour evolution theory *18*
Cancer stem cell theory *21*
Complementary and conflicting models *24*
Somatic evolution and genomic diversification 24
Epigenetic diversification 27
Cell identity and the value of single gene-variants 29
Cell extrinsic contributions in adaptation, selection and convergence 30
Supra-cellular dimensions and causality 33
Résumé *36*
PRECISE MANAGEMENT OF AML 36
Disease delineation *38*
Disease stratification *39*
Predictions and risk-adapted management *40*
Targeted therapy *41*
FLT3-LM AML and the challenges of precision *42*
SUMMING UP SO FAR: THEORIES, PRECISION AND FRICTION 43
AIMS **45**

METHODOLOGICAL CONSIDERATIONS **46**

STUDY DESIGN 46

SAMPLE MATERIAL AND SAMPLE MANAGEMENT 47

GENOMIC CHARACTERISATION 48

PHENOTYPIC CHARACTERISATION 50

Differential gene expression analysis *50*

Mass and flow cytometry *51*

FUNCTIONAL CHARACTERISATION 52

Drug sensitivity and resistance testing *52*

Repopulation assays *53*

STATISTICAL CONSIDERATIONS 53

RESULTS AND DISCUSSION **54**

FLT3-LMS – CAUSE OR EFFECT? 54

SOMATIC DIVERSIFICATION AND A SELECTION-CENTRED PERSPECTIVE 56

THE CANCER CELL – A CONFINED ENTITY OR A POTENTIAL PROPERTY? 57

Intra-leukaemic heterogeneity and clonal dominance *58*

Latency and emergence of secondary malignancies in relation with non-genotoxic therapy *58*

Spontaneous remissions, late recurrences and donor cell leukaemia *59*

Persistence of cell populations characterised by oncogenes in healthy individuals *60*

LEUKAEMOGENEIC TRANSITIONS AND LIMITATIONS OF SOMATIC MUTATION THEORY 61

PRECISION MEDICINE AND THE COMPLEXITY OF BIOLOGICAL SYSTEMS 63

Possible limitations with regards to molecular delineation of AML and AML subtypes *65*

Molecular targeted therapy and opportunities and challenges in AML *65*

Knowledge, predictions and action in AML *67*

THE IMAGINARY OF PRECISION MEDICINE AND UNINTENDED CONSEQUENCES 68

FUTURE PERSPECTIVES **70**

CONCLUSIONS **71**

REFERENCES **73**

SUPPLEMENTARY MATERIAL **125**

Supplementary table 1 – PMID result from literature search *125*

Supplementary table 2 – WHO classification system *126*

Supplementary table 3 – ELN risk stratification system *127*

Supplementary figure 1 – Serial sampling and exome sequencing in Beat-AML *128*

Supplementary figure 2 – Comparing bone marrow and peripheral blood samples in Beat-AML *133*

PAPER I-III **134**

Introduction

Preface

This dissertation explores the biological heterogeneity of acute myeloid leukaemia (AML). As such, it presents the findings of my doctoral research within and inside of one of the many highly specialised fields of biomedicine. Specifically, we – my co-authors and I – have studied details of the genetic and phenotypic diversity in AML in which the *FLT3* gene is length mutated, i.e., *FLT3*-LM AML.

At the same time, this arguably small world of biological heterogeneity and complexity of a quite rare disease is connected to the much larger world of cancer research and cancer treatment, and – as will become clear – to general conceptual frameworks for cancer and general visions of what medicine is and should become. Specifically, the vision of *precision medicine* – in the cancer field, known as precision oncology – is not only a matter of political imagination and discourse but also of concrete choices in everyday biomedical research and clinical practice. In the course of my dissertational work, I have come to see ever more connections between the findings in the biomedical laboratories and the larger scientific and political issues. These connections go in both directions. Notably, "small" empirical findings may have broad theoretical implications. In this PhD project, I found it necessary to engage with both of these worlds in order to make sense of *FLT3*-LM AML. The empirical characterisations that we present in Paper I-III called for theoretical reflection and interpretation that went beyond the genre of biomedical research papers (and thus were included in this synthesis); however, these reflections and interpretations also appeared to have implications for how to understand AML, how to understand cancer, and how to evaluate the current imaginaries of precision oncology. This synthesis – the part of the dissertation that introduces and discusses the three included research papers – is my attempt to make sense in writing of the nexus of issues defined by *FLT3*-LM AML at the smaller end and precision oncology at the larger. I will do so in a zigzagging motion. First, some introductory words are needed about cancer and precision oncology.

Worldwide, cancer is a major cause of health impairment and premature death. With the rise in life expectancy across the globe, cancer incidence and mortality rates are estimated to increase substantially in the decades to come (Global Burden of Disease Cancer Collaboration, Fitzmaurice et al. 2018, Global Burden of Disease Causes of Death Collaborators 2018). Efforts aimed at improving clinical management of cancer are extensive (Eckhouse, Lewison et al. 2008, van de Loo, Trzaska et al. 2012). At the heart of this exertion translational cancer research (Cambrosio, Keating et al. 2006) and the imaginaries of precision medicine and precision oncology are taking

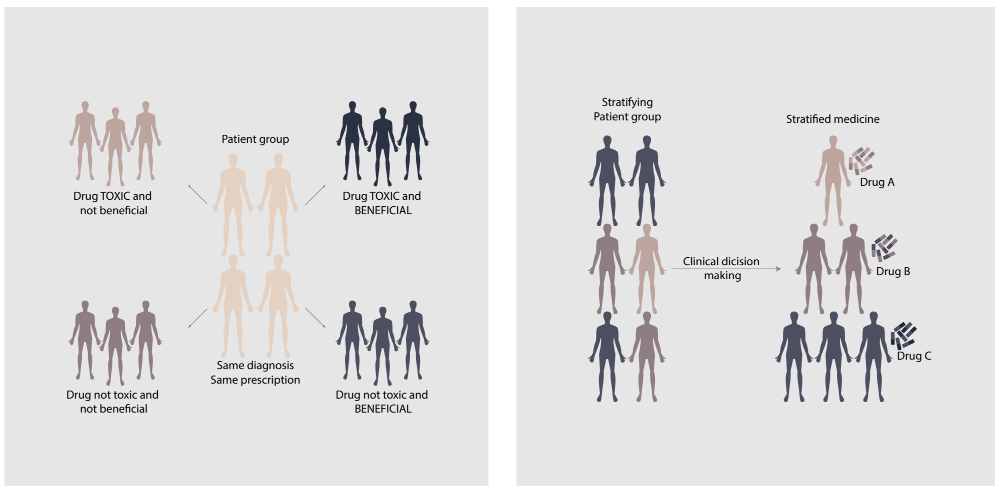

Figure 1: Precision medicine is a relatively new term overlapping and gradually replacing the preceding term "personalised medicine". According to the Precision Medicine Initiative launched by Barak Obama in 2015 precision medicine is "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person". The approach is founded on the assumption that interindividual heterogeneity result in suboptimal utility of medical interventions. Through disease stratification and tailoring of medical care outcomes can improve. The illustrations are adapted from various online sources based on the google image search "precision medicine".

shape and gaining traction (Hamburg and Collins 2010, National Research Council (US) 2011, Mirnezami, Nicholson et al. 2012, Collins and Varmus 2015, Celis and Heitor 2019).

Precision oncology adheres to the prevailing conceptual understanding of what cancer is: a clonal disease, caused by acquisition and accumulation of genetic alterations in cells, ultimately resulting in disruption of normal cell function (Nowell 1976, Vogelstein and Kinzler 1993, Hanahan and Weinberg 2000, Garraway, Verweij et al. 2013). On the premise that these molecular events are patient specific and at the core of the causality of cancer precision oncology proposes a change of cancer management along two dimensions: i) from groups to individuals and ii) from morphological to molecular classification. Through identification of causally contributing molecular mechanisms the goal is to enable precise disease categorisation, prediction, prevention, early detection and targeted treatment; providing the right treatment to the right patient at the right time (Mirnezami, Nicholson et al. 2012, Tsimberidou, Eggermont et al. 2014, Collins and Varmus 2015, Ashley 2016) (Figure 1).

The integration of precision oncology related approaches in standard patient care is an ongoing process, resulting in shifts in diagnostic thresholds, formation of novel disease subcategories and adaptation of new treatment strategies (Jameson and Longo 2015). Currently, however, only a limited fraction of cancer patients is estimated to benefit from this line of approaches (Marquart, Chen et al. 2018). Based on the limited progress so far some investigators and clinicians have even challenged the validity, utility and sustainability of precision oncology all together (Prasad 2016,

Prasad, Fojo et al. 2016, Marquart, Chen et al. 2018). The tension between the current status of precision oncology and the optimism related to future benefits of this strategy is an important motivation for this work. In the following sections I will give a brief outline of the emergence of precision medicine and precision oncology. Next, I shall introduce *FLT3*-LM AML as a rare but severe disease that is important in its own right and also an interesting case for discussing conceptual frameworks of cancer. Most of the synthesis is devoted to an in-depth discussion of various empirical features of *FLT3*-LM AML, including the results of Paper I-III. Finally, I shall zigzag back to the broader theoretical issues and discuss the possible implications of my (and others') findings for how to understand and manage cancer.

Precision medicine – tradition, evidence, reason and ambition

The advancement towards increased precision in medicine and oncology can be seen as a continuation of the direction modern medicine has had since its conception (Le Fanu 2000). A recent analysis of ancient Hippocratic texts identified that inter-individual heterogeneity was recognised already 2500 years ago. This suggests that individually tailored treatment and medical care always has been a fundamental feature of applied medicine (Konstantinidou, Karaglani et al. 2017). It is, however, only throughout the last two centuries molecular mechanisms underlying this inter-individual heterogeneity have begun to be revealed. Technological progress has allowed a gradual increase in resolution in the exploration of both human physiology as well as pathology. Disease classification systems as well as clinical practices have evolved in close relationship with methodological advancements. This development is characterised by gradual shifts in dimensionality from the clinical and macro-anatomical organisation and understanding of human maladies to tissue centred approaches, followed by increasing attention on cells and subcellular components as the origin of pathology (Keating and Cambrosio 2001).

The concept of "molecular" disease was first put forward in 1949 by Pauling and colleagues in the Science paper "Sickle Cell Anemia, a Molecular Disease". The authors hypothesised the genetic basis for the condition, and experimentally explored the aberrant protein product responsible for erythrocyte "sickling" (Pauling, Itano et al. 1949). In the decades that followed and up until the present genotype-phenotype relationships have been confirmed to account for a myriad of human traits and disease phenotypes (Buniello, MacArthur et al. 2019). The idea of precision medicine gradually emerged from this body of knowledge. It was, however, in relation to the planning and execution of the "Human Genome Project", formally commenced in 1990, that the vision of precision medicine was truly articulated (Collins 1999). The Human Genome Project was a milestone in the development of the implicit idea of "precision medicine" into a recognizable sociotechnical imaginary: a shared vision, ambition and commitment, co-created and co-

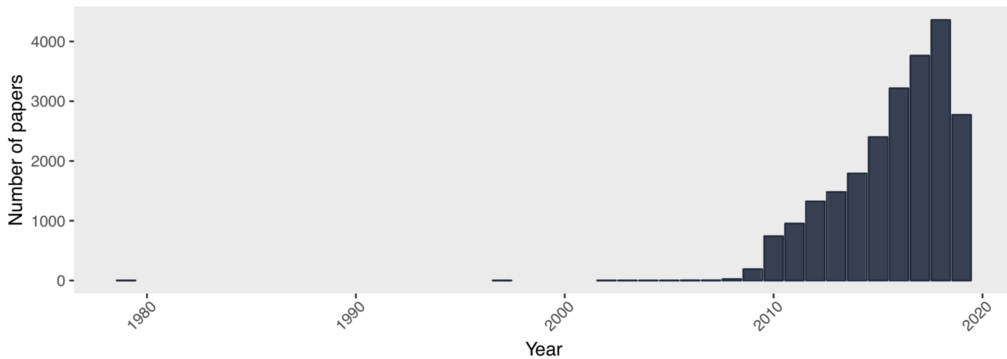

Figure 2: Histogram of the temporal distribution papers associated with the term "Precision medicine" in the search-engine Pubmed (10.07.2019).

maintained by experts and policy makers (Jasanoff and Kim 2015, Tarkkala, Helén et al. 2019). The goal of the "Human Genome Project", providing a complete sequence of the human genome, was ambitious and required considerable financial and intellectual investment. The legitimacy of this publicly funded venture was rationalised through postulations of significant scientific, medical, and societal advancements (National Research Council (US) 1988). Francis Collins[1] put it like this: "Scientists wanted to map the human genetic terrain, knowing it would lead them to previously unimaginable insights, and from there to the common good. That good would include a new understanding of genetic contributions to human disease and the development of rational strategies for minimising or preventing disease phenotypes altogether" (Collins 1999)[2].

Since 1999 the imaginary of precision medicine has matured and expanded beyond its initial scope to propose fundamental changes not only in how diseases are to be managed but also to be categorised and understood. In 2011 the National Research Council (US) released the report "Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease". Here the authors commend the development of a new taxonomy of human diseases, predominantly based on intrinsic biology and causal molecular disease mechanisms rather than signs and symptoms (National Research Council (US) 2011). The term "precision medicine" has since then rapidly been integrated in the biomedical and biotechnological scientific literature (Figure 2) as well as the political, regulatory and public discourse (Blasimme and Vayena 2016). The uptake has been substantially intensified by the launch of the "Precision Medicine Initiative" by Barak Obama in 2015, aimed at accelerating the translation of biomedical science to improved clinical outcomes (Collins and Varmus 2015).

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[1] Francis Collins was director of the National Human Genome Research Institute from 1993-2008 and is currently the director of the National Institutes of Health. US.

[2] Quote from the 1999 Shattuck lecture, titled: "Medical and societal consequences of the human genome project".

Precision oncology – expectations and realisations

Considered a genetic and molecular disease cancer served as an example of the hypothesised future significance of the Human genome project as well as the transition towards a molecular based disease taxonomy (National Research Council (US) 1988, National Research Council (US) 2011). Precision medicine in relation to cancer management has been characterised by a strong emphasis on inter-individual variability of genes, and is often referred to as genomics-driven cancer medicine (Garraway, Verweij et al. 2013). Medical strategies related to precision oncology are profoundly tied to postulations of genetic causality in cancer development. The idea of a monoclonal origin of cancer suggest that the cellular mass of an individual tumour share molecular characteristics involved in pathogenesis. Observations of cellular dependency of mutated or aberrantly expressed gene-products for both initiation and maintenance of malignant phenotypes support this idea and led to the postulation and experimental verification of "oncogene addiction" (Weinstein 2002). This provided a strong rational for the possibility of classifying various cancers with respect to their molecular origin, as well molecular targeted treatment strategies. The feasibility of this approach was confirmed in the early 2000s based on several unprecedented clinical success stories, including molecular targeted therapy in chronic myeloid leukaemia (Deininger, Buchdunger et al. 2005) gastrointestinal stromal tumours (DeMatteo 2002) and a molecular defined sub-group of breast cancer (Slamon, Leyland-Jones et al. 2001).

Identification of shared molecular "drivers" in cancer cells originating from discrete cell-types and diverse tissues led to the hypothesis that this approach may be scalable, perhaps even to all cancers and all cancer patients (Tsimberidou, Eggermont et al. 2014). Instead of managing cancers in accordance with their macro-and microanatomical origin treatment could be guided by genomic profiling (Garraway, Verweij et al. 2013). Recently, therapeutic compounds based on molecular defined indications rather than tissue or histology, such as pembrolizumab, were subject to regulatory approval[3] (Lemery, Keegan et al. 2017, Scott 2019). This development can be seen as a sizeable stride towards making such an approach become standard of care.

While tissue agnostic indications strongly enforce the implementation of molecular profiling of all cancer patients it has been challenging to demonstrate that broad genetic testing followed by rationally selected therapeutic compounds generally lead to superior outcomes compared to current evidence-based practices (Le Tourneau, Delord et al. 2015, Stockley, Oza et al. 2016, Massard, Michiels et al. 2017, Rodon, Soria et al. 2019, Rothwell, Ayub et al. 2019). Experience from

[3] In 2017 U.S. Food and Drug Administration (FDA) provided approval of a programmed death 1 (PD-1) inhibitor (pembrolizumab) for patients with microsatellite-instability–high or mis-match-repair–deficient solid tumours. This was followed by the authorisation of a tropomyosin kinase receptor inhibitor (larotrectinib) for cancer patients with neurotrophic receptor tyrosine kinase (NTRK) gene fusions regardless of anatomical origin.

multiple trials as well as general estimates suggest that currently only a small percentage of cancer patients with advanced stage disease are eligible and will benefit from genome-informed therapy. Furthermore, the magnitude of clinical benefit that can be attributed to biomarker matched interventions is sobering. So far, it is a matter of additional months of life (Marquart, Chen et al. 2018, Sicklick, Kato et al. 2019), rather than years or decades, as has been achieved in chronic myeloid leukaemia, gastrointestinal stromal tumours and some patients with breast cancer (Slamon, Leyland-Jones et al. 2001, DeMatteo 2002, Deininger, Buchdunger et al. 2005).

The limited benefit of precision oncology may in part be accounted for by lack of knowledge as well as restrictions in technology, availability of therapeutic compounds and investigation in suboptimal study populations. Discovery of novel targets, development of better technological solutions, increased availability of therapeutic compounds, improved clinical infrastructure, and therapeutic repositioning to earlier disease stages may all contribute to further progress of this approach. However, more than 20 years have passed since precision oncology related approaches were first projected to result in substantial benefit (Collins 1999). Based on the current discrepancy between the promises and 20 years of experience with precision oncology it seems timely to re-explore the theoretical foundations of precision oncology.

The case

My dissertation explores certain features of acute myeloid leukaemia (AML). Cancers of the haematopoietic system are relatively infrequent (Global Burden of Disease Cancer Collaboration, Fitzmaurice et al. 2018). AML, for example, has an age-standardised incidence rate estimated to be 3.62 per 100 000 (Sant, Allemani et al. 2010). Still, the discipline of haemato-oncology is important as it pioneered and set directions for oncological research and medical oncological practice since the early 1950s. The first randomised comparative clinical trial was performed in leukaemia (Frei, Holland et al. 1958), and both chemo-therapeutics, combinational regimes (Chabner and Roberts 2005), adaptive cell- and immune-therapy (Singh and McGuirk 2016) and more recently gene-therapy (Rosenbaum 2017) were all first explored and developed in patients with cancers of the blood. The first major breakthrough in the application of precision therapy also derives from haemato-oncological practice with the introduction of all-trans-retinoic-acid in acute promyelocytic leukaemia (Wang and Chen 2008), a therapeutic strategy developed over 30 years ago. Subsequently, the proof of concept for molecular targeted therapy was obtained for chronic myeloid leukaemia. The molecular framing of this disease, based on the identification of the Philadelphia chromosome and the functional characterisation of the BCR-ABL fusion protein (Nowell and Hungerford 1961, Deininger, Buchdunger et al. 2005) resulted in the development of pharmaceutical agents that specifically target the oncogene protein-product which gave remarkable

improvements of outcome for this patient group (Deininger, Buchdunger et al. 2005). Haemato-oncological diseases have also frequently provided paradigmatic empirical examples to support prevailing theories of cancer, including somatic mutation theory, tumour evolution theory (Nowell 1976), and cancer stem cell theory (Bonnet and Dick 1997). Furthermore, the diversity and contrasts within haemato-oncology makes it a hugely interesting field. Above all, the contrast between the success of targeted therapy in acute promyelocytic leukaemia (Wang and Chen 2008) and chronic myeloid leukaemia (Deininger, Buchdunger et al. 2005) and the lack of sizeable therapeutic progress in AML (Short, Rytting et al. 2018) poses both scientific and clinical challenges. In this regards *FLT3*-LM AML is of particular interest. Descriptively and functionally *FLT3*-LM AML shares some resemblance to *BCR-ABL* positive chronic myeloid leukaemia. As myeloid malignancies AML and chronic myeloid leukaemia derive from related cell populations, and they both appear to be driven by genetic aberrations that result in constitutive activation of tyrosine kinases. Encouraged by the success of BCR-ABL targeted therapy, FLT3 was very early singled out as an attractive therapeutic target in AML (Gilliland and Griffin 2002, Kelly and Gilliland 2002, Levis and Small 2003, Stirewalt and Radich 2003). The FLT3 protein is druggable and throughout the last two decades considerable effort has been devoted to the development of therapeutic agents specifically inhibiting the activity of FLT3. Multiple small molecule inhibitors have been demonstrated to induce anti-leukaemic responses in preclinical models as well as in clinical trials[4] (Engen, Wergeland et al. 2014). Despite this fact FLT3 inhibition has not been able to provide more than transient anti-leukaemic responses and marginal clinical benefits, both when administered as monotherapy as well as in combination with chemotherapeutic agents (Kindler, Lipka et al. 2010, Stone, Mandrekar et al. 2017, Cortes, Khaled et al. 2019). In addition to its importance to current and future AML patients, the resistance of *FLT3*-LM AML to molecular clinical approaches makes it a challenging test case for precision oncology.

[4] Response criteria in AML: Complete remission is defined by reduction of bone marrow blasts to less than 5% as well as recovery of haematological function. Additional criteria include absence of circulating blasts, Auer rods and extramedullary disease. Complete response without minimal residual disease further requires clearance of leukemic cells as assessed by a genetic test or by flow cytometry. Complete remission with incomplete haematological recovery is achieved when the criteria of complete remission are fulfilled but signs of bone marrow failure persist. To fulfil the criteria of morphologic leukaemia-free state bone marrow blasts must be less than 5%, while bone marrow cellularity is at least 10%, in the absence of circulating blasts, Auer rods as well as extramedullary disease. A partial remission can be considered achieved by reduction of bone marrow blast percentage by at least 50% as compared to the blast percentage prior to treatment. In trials stable disease may also be included as a response criterion. It is applied when neither of the criteria above are met but the disease is stable over a time period of at least 3 months (Dohner, Estey et al. 2017).

Figure 3: The histograms summarise the AML literature search as described in the method section. The word cloud represents the most cited FLT3 scientific literature. In detail 23.04.2019 I queried the PubMed database with the phrase FLT3[AB] OR FLT3-ITD [AB] and retrieved search results from 1991 and up to 2019 identifying a total of 5286 unique publications. I retained papers cited more than 50 times in PMC articles, resulting in 350 papers. I attained the abstracts from these publications and generated a text file I further quantitatively assessed for repetitive words and phrasing. The word cloud is a graphical representation of the 200 words/phrases most frequently appearing in the abstracts after subtracting common "stopwords" (words with near to zero information value like «and», «the», «also», ect). The size and color of the word indicate the frequency of which they appear, with «FLT3» being identified 957 times in the text. The analysis demonstrates that the research literature on FLT3 is largely focused on AML. The emphasis on somatic mutation theory is accentuated by the number of times mutations or mutation is mentioned (889) as well as the frequent occurrence of other gene-names.

Methodological approach of the synthesis part of the dissertation

As is usual for medical doctorates at the University of Bergen, I have chosen to address my research questions through a set of (three) research papers that address specific empirical matters, and a synthesis that interprets the empirical findings in light of a broader theoretical discussion. In my case there has been a need not only to discuss the findings within what might be called the prevailing research paradigm in which the individual papers are being published, but also to engage in a critical discussion of this paradigm and discuss the significance of my findings for this critique, and vice versa. Accordingly, for the synthesis part of the thesis, I have made an attempt not only to summarise and review the main knowledge claims at the forefront of haemato-oncological research but also to develop a systematic characterisation of this research literature as a discourse, in order to be able to criticise it with some rigour. In this section I will describe the methodological approach used in the literature study underlying the synthesis.

A key premise of the vision of precision medicine is that its medical practices are expected to be in close correspondence to the understanding of pathophysiological mechanism. This is what is envisioned to make precision medicine precise. For the literature study underlying the synthesis, I

have examined the conceptual emphasis with regards to AML pathogenesis in haemato-oncological research. Specifically, I reviewed the most cited scientific literature on AML published since the year 2000[5] (Figure 3). Assuming that the number of citations reflects scientific impact in the AML research field as well as clinical haematology, I retained papers cited more than 100 times and procured these publications for qualitative assessment. The PubMed IDs (PMID) for the final list of papers is provided in supplementary table 1. I categorised the papers with respect to thematic content and in accordance with explicitly or implicitly articulated conceptual frameworks rationally anchoring the knowledge generated. I further identified articulations related to the clinical applicability of the research with particular attention on precision medicine related approaches. A total number of 254 publications were included in the analysis after exclusion of papers not directly related to the AML research field or papers with a predominant focus on methodological development.

The body of literature was strongly dominated by biomedical reports, with a strong emphasis on translating biomedical knowledge to medical practice. Papers focusing on molecular profiling of AML were prevailing, followed by experimental papers exploring basic biology or drug development in pre-clinical AML model systems. Relatively few papers reported outcomes from clinical trials. The literature selection further included several review papers in addition to a couple of epidemiological reports and meta-analyses.

The remaining introduction of this thesis is structured in accordance with main themes identified across the literature analysis. I start by giving a brief introduction to AML and the *FLT3* gene and the FLT3 protein. I precede by presenting an outline of the prevailing theoretical frameworks of AML pathogenesis as identified in the literature, incorporating observational and experimental data from the study of *FLT3*-LM AML. I then provide a summary of the principal precision medicine related approaches in AML and I explore how they relate to theoretical assumptions.

Acute myeloid leukaemia

A constant move towards increased resolution is clearly evident in the study and management of cancers of the blood. Leukaemia was first described as a distinct clinical entity in the middle of the 19th century. Initially the disease was recognised based on autopsy findings and later by symptoms

[5] In detail the 02.05.2019 I queried the PubMed database with the phrase (acute myeloid leukaemia[Title/Abstract]) OR (acute myelogenous leukaemia[Title/Abstract]) OR (acute myelogenous leukemia[Title/Abstract]) OR (AML[Title/Abstract]) OR (acute myeloid leukemia[Title/Abstract]) and retrieved search results from the period 2000 to 2019, identifying a total of 32703 unique entries. Median number of PubMed Central (PMC) article citations per paper was 2, with a total of 8787 papers with no citations and 320 papers cited more than 100 times. The maximum number of PMC article citations was 1606.

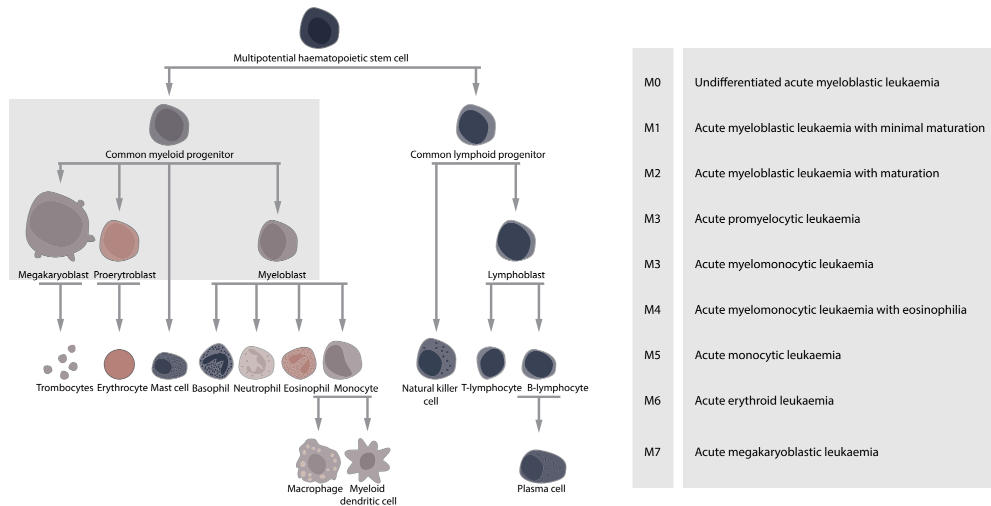

Figure 4: To the left a classical representation of the hierarchical organisation of the haematopoietic system. The cell types hypothesised to represent the cellular origin of AML are highlighted. To the right an overview the French-American-British (FAB) classification system of AML, sub-classifying AML according to cytomorphological features.

and distinct clinical and biochemical observations. Based on this level of resolution leukaemia could be divided in two subgroups: an acute, storming and rapidly lethal form of the disease, contrasted by a more indolent, chronic form of leukaemia. Only when microscopic assessment of the blood was available by the middle of the 19th century did one approximate the nature of leukaemia as a neoplastic condition originating in the haematopoietic system. Based on morphological distinctions leukaemia was subjected to additional stratification: leukaemia originating from cells committed to the myeloid lineage of the haematopoietic system as opposed to leukaemia deriving from the lymphatic cell lineages (Kampen 2012). Founded on morphological and cytochemical attributes further sub-classifications followed, and by 1976 three groups of acute lymphoblastic leukaemia and six distinct morphological subgroups of AML were proposed (Bennett, Catovsky et al. 1976) (Figure 4). Simultaneously, the clonal origin of leukaemia was suggested, and studies explored the relationships between disease phenotype, cell of origin and genetic alterations (Nowell 1976). This perspective gave rise to a shift from a descriptive, morphologically based system for organising the different sorts of leukaemia to a more pathophysiological and functionally founded organisational structure, frequently based on genetic aberrations (Harris, Jaffe et al. 2000, Vardiman, Thiele et al. 2009, Arber, Orazi et al. 2016).

In line with this development of increasing stratification, AML is currently understood as a collective term, congregating a heterogenous group of various acute blood cancers, morphologically and clinically characterised by interference with normal haematopoietic

organisation by expansion of myeloid haematopoietic progenitor cells at the expense of normal haematopoietic differentiation and maturation.

AML affects individuals of all ages, but predominantly presents late in life, at a median age of 72 years (Juliusson, Antunovic et al. 2009). Diagnosis usually follows identification of a relative increase of immature blood cells (blast) by examination of a bone marrow smear[6], obtained based on suspicion of haematopoietic involvement in clinical disease presentation. The disease history is usually brief, and the symptoms generally reflect the underlying process of expansion of immature cells and bone marrow failure. Common signs of presentation include anaemia, leukopenia and thrombocytopenia, occasionally accompanied with extramedullary disease involvement like tissue infiltration of leukaemic cells (Estey and Dohner 2006, Dohner, Weisdorf et al. 2015, Short, Rytting et al. 2018).

The natural course of AML is characterised by rapid disease progression which results in death, usually within a few weeks to a couple of months from the time of diagnosis (Oran and Weisdorf 2012). Outcome can be improved by therapeutic intervention, and current treatment options in AML include chemotherapy-based regimens, haematopoietic stem cell transplantation, and targeted treatment, as well as more lenient disease stabilising treatment plans and supportive care (Dohner, Estey et al. 2017).

FMS-like tyrosine kinase receptor 3

Despite the fact that the natural and uninterrupted advancement of AML is generally predictable, the response to available AML treatment regimens vastly differs, resulting in diversification of outcome in AML (Dohner, Estey et al. 2010, Dohner, Estey et al. 2017). The probability of discrete treatment responses corresponds to patient specific features like age and patterns of disease presentation (e.g. AML development following treatment with chemotherapy or AML progression from an antecedent myeloproliferative condition) (Appelbaum, Gundacker et al. 2006), but also relates to molecular characteristics like cytogenetic aberrations (Grimwade, Walker et al. 1998, Grimwade, Hills et al. 2010) and molecular genetic features (Schlenk, Dohner et al. 2008, Patel, Gonen et al. 2012, Papaemmanuil, Gerstung et al. 2016). The literature review revealed that this variability of outcome most commonly is interpreted as expression of discrete biological disease entities.

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[6] AML diagnosis requires a bone marrow blast count of 20% or more. Exceptions include cases where t(15;17), t(8;21), inv(16), or t(16;16) are identified. Under such circumstances identification of the translocation is sufficient for diagnosis (Dohner, Estey et al. 2017).

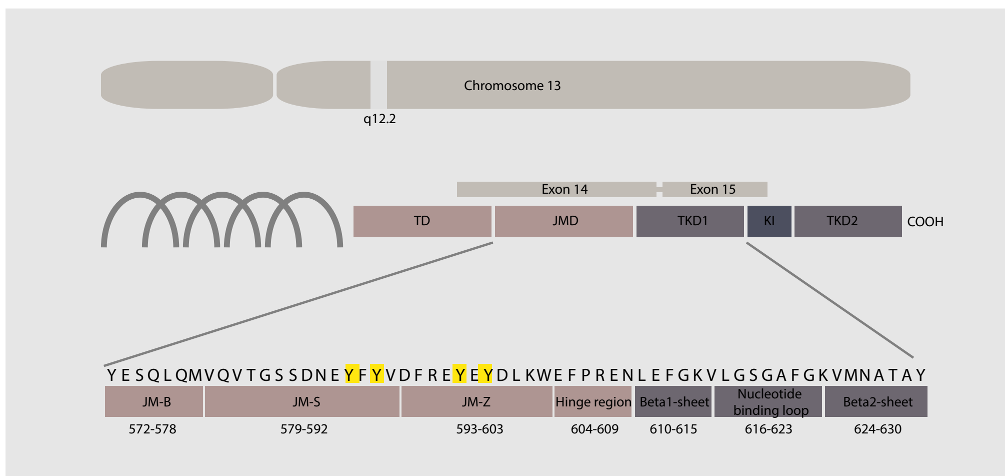


Figure 5: Illustration of the chromosomal location of the FLT3 gene and a graphical presentation of the structure of the region most frequently involved in internal tandem duplications according to functional domains. The figure is adapted from Schnittger, Bacher et al. 2012 and is also presented in paper I.

TD: transmembrane domain, JMD: juxtamembrane domain, TKD1: tyrosine kinase domain 1, KI: kinase insert region, TKD2: tyrosine kinase domain 2, COOH: C-terminus, JM-B: juxtamembrane binding motif, JM-S:, juxtamembrane switch motif, JM-Z: juxtamembrane zipper motif.

The single most frequently mutated gene in AML is *FLT3*[7] (Cancer Genome Atlas Research Network 2013, Tyner, Tognon et al. 2018), and the mutation status of this gene is well recognised to predict disease outcome in AML (Daver, Schlenk et al. 2019). This is also the single most frequently mentioned gene in the literature selection, underscoring the position of this gene in the AML research field.

The *FLT3* gene and the corresponding protein product was first discovered, isolated and described in the early 1990s by two independent groups (Matthews, Jordan et al. 1991, Rosnet, Marchetto et al. 1991, Rosnet, Mattei et al. 1991, Rosnet, Schiff et al. 1993). The gene, located at chromosome 13, band q12.2, comprises a total of 96 982 base pairs, distributed across 24 exons and corresponding introns, with an estimated protein size of a total of 993 amino acid residues (Schnittger 2005). Structurally the gene shares resemblance to c*KIT*, *CSF1R* and *PDGFR*, and it is classified as a member of the class III tyrosine kinase receptor family. This is a group of trans-membrane cytokine/growth factor receptors, characterised by an extracellular ligand-binding domain and an intra-cellular tyrosine kinase domain (Figure 5 and Figure 6).

Gene and protein expression studies have demonstrated that *FLT3* predominantly is expressed in haematopoietic tissue, including early progenitors of both myeloid and lymphatic linages (Matthews, Jordan et al. 1991, Rosnet, Schiff et al. 1993), suggestive of a ligand dependent role in

[7] Alternative names for FLT3 include CD135, FLK2, STK1, and ENSG00000122025.

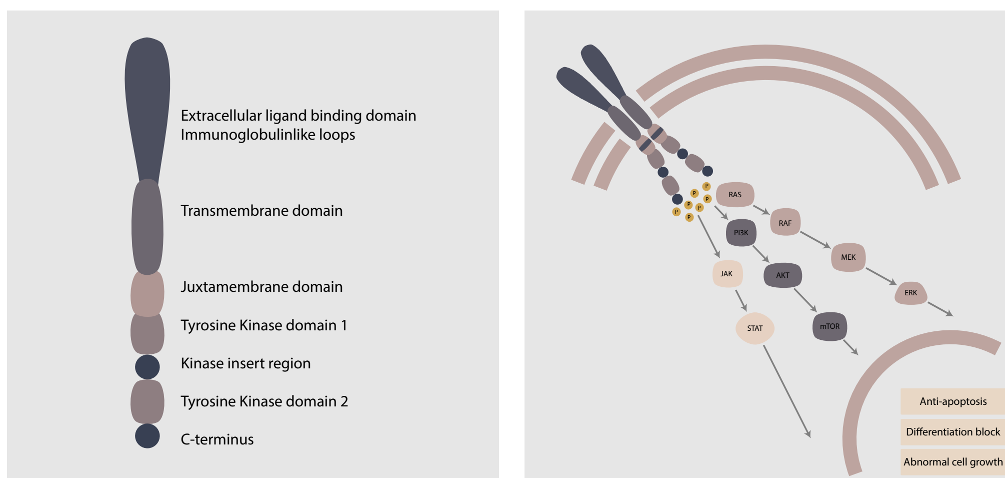


Figure 6: An illustration of the FLT3 receptor showing the various protein regions, the location of the internal tandem duplication of genes within the juxtamembrane domain as well as downstream signalling pathways. The figure is adapted from Figure 1 in Swords, Freeman et al. 2012.

PI3K: phosphatidylinositol 3-kinases, AKT: AKT Serine/Threonine Kinase, mTOR: Mechanistic Target Of Rapamycin Kinase, RAS: RAS Proto-Oncogene, RAF: RAF Proto-Oncogene Serine/Threonine-Protein Kinase, MEK: Mitogen-activated protein

growth and differentiation processes (Lyman, James et al. 1993). This was confirmed by functional assessment, demonstrating that FLT3 is an enzyme involved in the maintenance of adult haematopoiesis. Binding of its ligand, FLT3-ligand, is normally followed by a conformational change, and subsequent activation of the intrinsic tyrosine kinase domain. This results in initiation of multiple intra-cellular signalling pathways through phosphorylation of mediator molecules. FLT3 signalling has been shown to play a key role in the maturation of monocytes, macrophages and dendritic cells (Tsapogas, Mooney et al. 2017). FLT3-ligand has further been shown to be upregulated under conditions of haematopoietic stress, suggesting that FLT3 signalling is a central mediator of haematopoietic expansion under conditions of demand (Lyman, Seaberg et al. 1995, Wodnar-Filipowicz, Lyman et al. 1996).

FLT3 was already in 1992 suggested to be involved in leukaemogenesis when high expression was found in most AML cases, in a substantial fraction of acute lymphoblastic leukaemia of both T- and B-cell origin, as well as in chronic myeloid leukaemia blast crisis (Birg, Courcoul et al. 1992). In 1996 Nakao and colleagues made a key discovery while studying *FLT3* mRNA expression in adult leukaemia patients. They discovered that 5 of 30 examined mRNA specimens presented with transcripts that exceeded the anticipated polymerase chain reactions amplified product. Sequencing of these transcripts revealed that they all contained length mutations (LMs) as partial internal tandem duplications (ITD), restrained to the juxtamembrane domain and the tyrosine kinase

domain 1[8] (Nakao, Yokota et al. 1996). This finding was validated in larger cohorts of AML, demonstrating that *FLT3*-LMs occur in a considerable proportion of adult AML and acute promyelocytic leukaemia patients (Yokota, Kiyoi et al. 1997). *FLT3*-LMs have subsequently been shown to be prevalent in both paediatric AML and acute lymphoblastic leukaemia, although with lower frequency than among adults (Xu, Taki et al. 1999). *FLT3*-LM have additionally been described in myelodysplastic syndrome (Meshinchi, Woods et al. 2001), and more recently in blastic plasmacytoid dendritic cell neoplasms (Pagano, Valentini et al. 2013).

Further examination of the *FLT3* gene in haematological cancers led to the identification of additional types of genetic aberrations, including several *FLT3* somatic non-synonymous/missense mutations, most frequently located in the tyrosine kinase domain 1 (TKD) region, with D835Y being the most frequent variant (Yamamoto, Kiyoi et al. 2001), but also described in the juxtamembrane domain (Reindl, Bagrintseva et al. 2006). Insertions, deletions and *FLT3* rearrangement have also been described (Deeb, Smonskey et al. 2014, Chung, Hou et al. 2017, Zhang, Paliga et al. 2018), and non-ITD, non-TKD *FLT3* mutations have recently been reported in myelodysplastic syndrome, myeloproliferative neoplasms (Higgins, Mangaonkar et al. 2018) and in acute lymphoblastic leukaemia (Zhang, Zhang et al. 2019).

Theoretical frameworks in AML

The literature analysis performed for the synthesis part of this dissertation indicated that current understanding of AML is founded on several intersecting and complementary theories of cancer. On the whole, the explanatory narratives identified contained details specific to AML. Still, as one might expect, they also strongly resembled prevailing general theoretical frameworks of cancer, including somatic mutation theory, tumour evolution theory, and cancer stem cell theory. In the following pages, I shall describe and discuss how these frameworks are included, presented and interpreted in the empirical knowledge base of AML, and in particular *FLT3*-LM AML, with respect to disease causality, leukaemic development and cellular origin. Before entering into these details, however, I present a brief historical account of the general theoretical frameworks (or "models") of cancer.

The somatic mutation theory was first proposed by the German biologist Theodor Boveri in the seminal paper "Frage der Entstehung maligner Tumouren", published in 1914 (Boveri 2008). Based on observations of the development of sea urchin eggs he put forward a theory suggesting that cancer formation is clonally derived from a single somatic cell and results from genetic

[8] ITDs in the *FLT3* gene frequently also involve small insertions. I thus refer to *FLT3*-LMs: comprising *FLT3*-ITDs, insertions in *FLT3* as well as *FLT3*-ITDs in addition to extra nucleotides.

damage. Tumour evolution theory, the idea of cancer as a disease that develops over time in multiple steps, was later articulated and conceptualised by the Finnish historian and architect Carl O. Nordling in a paper published in the British Journal of Cancer in 1953. The theory was founded on analysis of epidemiological data with focus on the relationship between age distribution of cancer presentation and exposure of mutagens. The result suggested that cancer is the outcome of a process that takes several years and sometimes even decades to develop to clinically overt disease. These two ideas, somatic mutation theory and tumour evolution theory, only really gained traction after the identification of specific recurring oncogenes, including the Philadelphia chromosome (Nowell and Hungerford 1961). A unification of the two models was subsequently put forward by Peter Nowell in the landmark paper "The clonal evolution of tumor cell populations", published in Science in 1976. Based on the previously developed theoretical frameworks and supported by his own observations and experimental results Nowell synthesised that cancer has a monoclonal origin and results from a stepwise accumulation of somatic mutations, ultimately giving rise to a malignant phenotype (Nowell 1976). At the same time theories of the cellular origin of cancer were put forward. Since cancer evolves over time and through progressive steps John Cairns postulated that the cell of origin must be long lived and characterised either by pre-existing or acquired self-renewal properties. Only mutations in persistent long-lived cells endure and only mutations in cells which divide and produce progeny emerge (Cairns 1975).

From the reviewed literature it was possible to identify and formulate a view that integrates the three models of somatic mutation theory, tumour evolution theory and cancer stem cell theory. While it would be too strong to characterise this view as a consensus, the reviewed literature was largely consistent with it. In this view, AML development is understood as the manifestation and gradual dominance of a novel aberrant cell population. This cell population is assumed to descend from a single haematopoietic stem or progenitor cell that as a result of sequential acquisition and accumulation of somatic mutations has progressed through a stepwise process resulting in the emergence of properties like differentiation block, autonomous proliferation and immortality (Figure 7).

In the following sections I will explore the knowledge base supporting these conceptual frameworks and I will interconnect them to the *FLT3*-LM AML literature with emphasis on the interpretation of *FLT3*-LMs as causal contributors in AML.

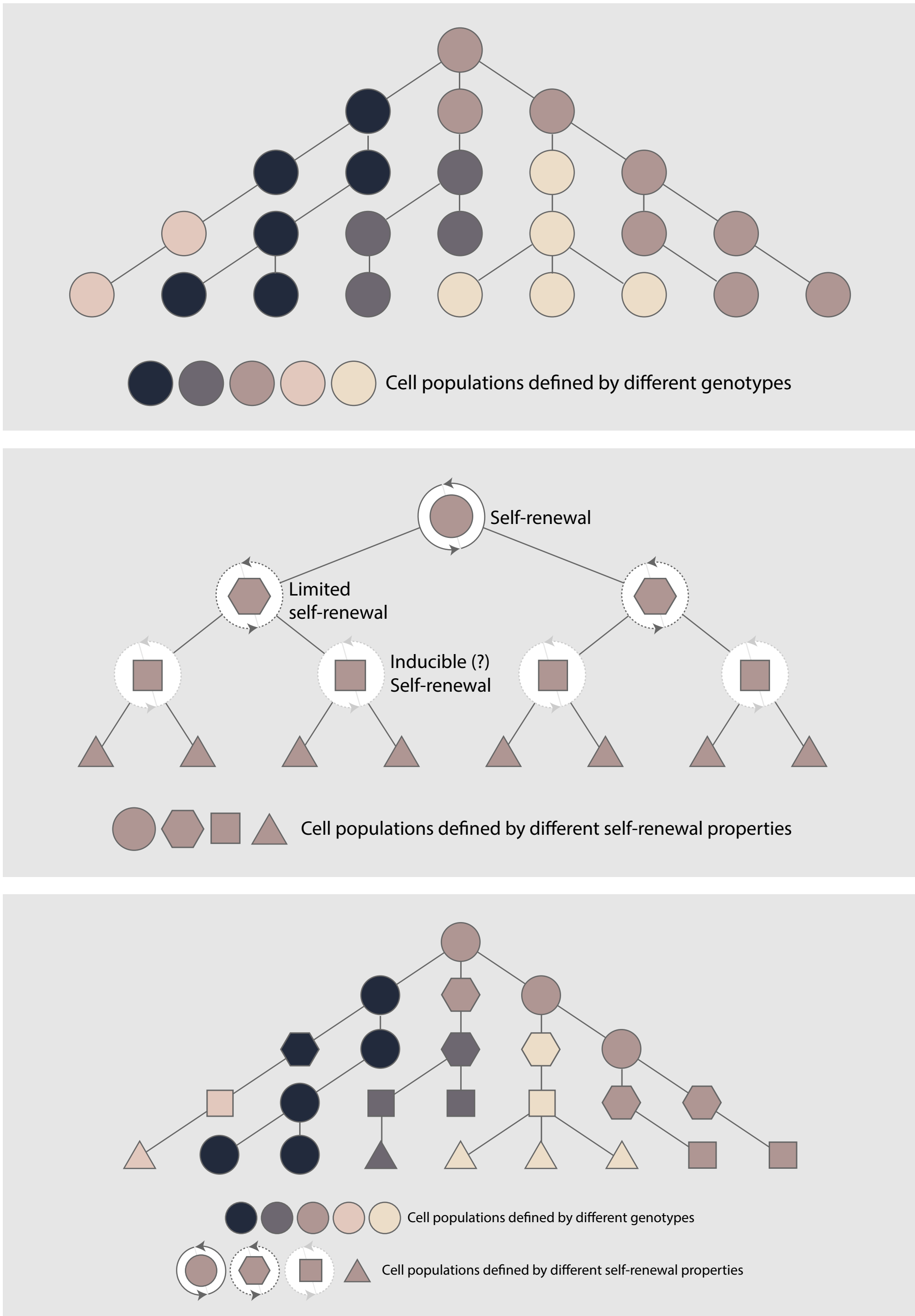


Figure 7: On the top classical model of tumour evolution theory depicting step-wise generation of variation through acquisition of somatic variants. In the middle classical representations of cancer stem cell theory, indicating gradual differentiation and heterogeneity with regards to self-renewal properties. On the bottom exemplification of how the cancer stem cell theory restrains the potential of tumor evolution. Only cells with self-renewal potential can persist and only cells that proliferate can populate a tumour.

Somatic mutation theory

The main assumptions of somatic mutation theory are i) that any cancer has a monoclonal origin and ii) that the acquisition of somatic mutations is the main cause of cancer development. The monoclonal origin of cancer was originally explored in studies of X-inactivation patterns in females where most cancers were shown to be characterised by a single X-inactivation pattern (Fialkow 1972, Vogelstein, Fearon et al. 1987). Subsequent identification of cytogenetic and molecular aberrations shared across all cells of individual tumours, including AML, provided further support of a shared unicellular ancestry (Kelly and Gilliland 2002, Mrozek, Heerema et al. 2004, Frohling, Scholl et al. 2005).

Recurrence of genetic aberrations of single genes has provided support to the idea of genetic causal contribution in cancer and in AML. Currently, more than 30 individual genes are recognised to be repeatedly mutated in AML, of which *FLT3* is the most commonly mutated. Combining single nucleotide variants and LMs, *FLT3* is mutated in up to 30-40% of adult AML patients (Cancer Genome Atlas Research Network 2013, Metzeler, Herold et al. 2016, Tyner, Tognon et al. 2018). Furthermore, most of the genes recurrently mutated are known to be important regulators of cell-identity and cell-fate, including genes involved in DNA methylation, cohesin complex formation, transcription, post-translational modification, as well as cell signalling enzymes and well known tumour suppressor genes (Cancer Genome Atlas Research Network 2013). The single most common group of genes mutated in AML are signalling genes, further strengthening the plausibility that *FLT3* mutations are causally implicated in leukaemogenesis (Cancer Genome Atlas Research Network 2013).

Relationships between genotypes and phenotypes, including outcome, have supported the genetic emphasis in cancer causality, proposing that genetic damage not only results in disease but in specific disease phenotypes. The detection of *FLT3*-LMs in primary patient material has been shown to be statistically associated with clinical, pathological as well as molecular features in AML. In detail positive *FLT3*-LM status has been associated with clinical features like leucocytosis and high bone marrow blast percentage at time of diagnosis (Kiyoi, Naoe et al. 1997, Kottaridis, Gale et al. 2001, Schnittger, Schoch et al. 2002). Positive *FLT3*-LM status has further been correlated with cytomorphological features along the myeloid differentiation axis (Thiede, Steudel et al. 2002) as well as with normal cytogenetics (Schnittger, Schoch et al. 2002, Thiede, Steudel et al. 2002) and mutations in genes like *NPM1* and *DNMT3A* (Cancer Genome Atlas Research Network 2013, Garg, Nagata et al. 2015, Tyner, Tognon et al. 2018). Expression of certain immuno-phenotypical markers (Munoz, Aventin et al. 2003, Rausei-Mills, Chang et al. 2008, Chauhan, Ihsan et al. 2013), intra-cellular signalling patterns (Irish, Hovland et al. 2004), gene expression signatures (Neben, Schnittger et al. 2005), micro RNA (Garzon, Volinia et al. 2008,

Whitman, Maharry et al. 2010) and long non-coding RNA profiles (Papaioannou, Nicolet et al. 2017), as well as chromatin accessibility configurations (Cauchy, James et al. 2015) have all been associated with positive *FLT3*-LM status. Identification of *FLT3*-LMs further indicate poor prognosis across several outcome indicators, including high likelihood of poor treatment response, high relapse rate, and inferior event-free, disease-free and overall survival (Kiyoi, Naoe et al. 1999, Abu-Duhier, Goodeve et al. 2000, Kottaridis, Gale et al. 2001, Meshinchi, Woods et al. 2001, Frohling, Schlenk et al. 2002, Schlenk, Dohner et al. 2008, Patel, Gonen et al. 2012).

Expression of *FLT3*-LMs in cellular model systems have demonstrated ligand independent dimerization and phosphorylation at the tyrosine residues, suggestive of gain-of-function properties (Kiyoi, Towatari et al. 1998, Kiyoi, Ohno et al. 2002). Introduction of *FLT3*-LM in growth factor dependent cell lines results in growth factor independency (Hayakawa, Towatari et al. 2000, Mizuki, Fenski et al. 2000, Zheng, Friedman et al. 2002). *FLT3*-LMs have further been shown to result in qualitative signalling alterations (Schmidt-Arras, Bohmer et al. 2009) through abnormal activation of at least three major intra-cellular pathways important for normal cell function: PI3K/AKT/mTOR, RAS/RAF/MEK/ERK/ and JAK/STAT (Gotze, Ramirez et al. 1998, Hayakawa, Towatari et al. 2000, Irish, Hovland et al. 2004, Kornblau, Womble et al. 2006, Choudhary, Brandts et al. 2007, Sanz, Burnett et al. 2009). The causal contribution of *FLT3*-LM has additionally been reinforced by demonstration of FLT3 dependency in *FLT3*-LM AML samples. Under *in vitro* conditions and in animal models over a dozen different tyrosine kinase inhibitors have shown anti-leukaemic effects, in support of oncogene addiction (Levis, Tse et al. 2001, Levis, Allebach et al. 2002, Weisberg, Boulton et al. 2002, Fathi and Levis 2011). This has further been supported by induction of clinical responses following treatment with FLT3 inhibitors in *FLT3*-LM positive patients (Smith, Levis et al. 2004, Stone, DeAngelo et al. 2005, Fischer, Stone et al. 2010). Disease progression characterised by addition of *FLT3* mutations conferring tyrosine kinase inhibitor resistance corroborates the conclusion that *FLT3*-LMs are important “drivers” in AML development (Smith, Wang et al. 2012).

Tumour evolution theory

While many recurrent cancers are strongly related to lifestyle, environmental exposure, or viral infections (Danaei, Vander Hoorn et al. 2005) AML is only weakly associated with identified risk-factors. Although smoking and exposure to radiation, alkylating chemotherapy, topoisomerase II inhibitors or substances like benzene are known to increase the likelihood of AML presentation, a history of such exposure can be identified only in a minor fraction of AML cases (Shallis, Wang et al. 2019). On the contrary several medical conditions, both congenital and somatic, are known to predispose for AML, including Fanconi anaemia, Diamond-Blackfan anaemia, trisomy 21,

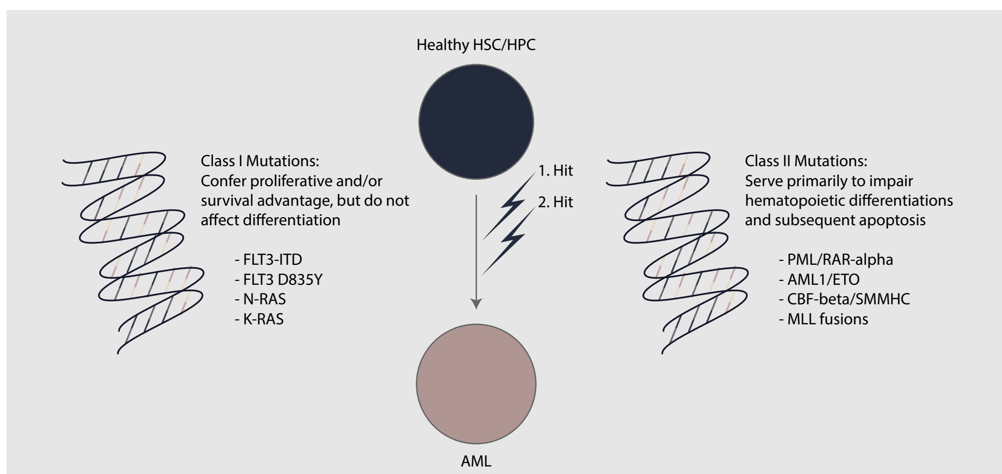


Figure 8: Graphical presentation of the "two hit" model as it was presented by Gilliland and Griffin in 2003. It illustrated a model of cooperativity between mutations conferring a proliferative drive and mutations resulting in impaired differentiation and apoptosis. The model was initially based on the observation of FLT3 activating mutations across all FAB groups as well as in most cytogenetic groups. They hypothesised that expression of the combination of these two classes of mutations would result in AML phenotypes. They further wrote that "the hypothesis has important implications in approach to novel therapies of AML, in that molecular targeting of both FLT3-ITD and fusion proteins involving transcription factors may improve outcome in AML". The figure is adapted from Gilliland and Griffin 2002.

HSC: haematopoietic stem cell, HCP: haematopoietic progenitor cell.

myelodysplastic syndrome, and myeloproliferative neoplasms (Deschler and Lubbert 2006). A multistep model of leukaemogenesis accounts for the latency between exposure and AML presentation. It further provides explanatory power, accounting for how germline variants or predisposing conditions increase the risk of AML, essentially as interrelated conditions evolving from the same underlying pre-leukaemic process.

In Nowell´s paper on tumour evolution he presented human leukaemogenesis as an example of a process characterised by relatively few "steps" (Nowell 1976), and until the 1990s the multistep genesis of AML remained controversial. Based on observations of persisting clonal cells in nonlymphocytic leukaemic remission Fialkow and colleagues established that also AML progress from a preleukaemic phase (Fialkow, Janssen et al. 1991). This was further supported by observations of persisting chromosomal aberrancies in AML remission (Miyamoto, Nagafuji et al. 1996), and more recently by residual cells characterised by AML related molecular somatic variants (Corces-Zimmerman, Hong et al. 2014, Ploen, Nederby et al. 2014).

In AML the prevailing theoretical fundament for understanding disease development is an expansion of tumour evolution theory which derives directly from the study of *FLT3*-LM AML. Gilliland and Griffin put forward a "2-hit" model of AML pathogenesis in a review paper titled "The roles of FLT3 in haematopoiesis and leukaemia" published in 2002. They suggested that AML development both necessitates and suffices two "hits": differentiation impairment (class 2

mutations) in addition to acquisition of proliferative and/or survival properties respectively (class 1 mutations), arguing for *FLT3*-LMs as a model for the later class of mutations (Gilliland and Griffin 2002) (Figure 8). The advent of next generation sequencing has rapidly enhanced the resolution of the genetical landscape of AML, expanding on the model. In 2008 and 2009 the first reports describing whole AML genomes were published (Ley, Mardis et al. 2008, Mardis, Ding et al. 2009), and over the last decade several large AML sample cohorts have been profoundly genetically characterised (Welch, Ley et al. 2012, Cancer Genome Atlas Research Network 2013, Tyner, Tognon et al. 2018). This has demonstrated that the number of recurrent somatic variants in AML is very low (average 2 - 5) in comparison with the numbers reported from a variety of solid cancers. This has provided support for Gilliland and Griffin´s postulation that as few as two mutations can suffice for AML initiation (Welch, Ley et al. 2012, Cancer Genome Atlas Research Network 2013).

Identification of recurring mutational patterns in AML involving *FLT3*-LMs and mutations or structural rearrangements categorised as class 2 mutations has further strengthened the hypothesis. This indicates that mutated *FLT3* gene-products synergise with other variant gene products such as *NPM1* and *DNMT3A* or with fusions such as *PML-RARA* in leukaemogenesis (Renneville, Roumier et al. 2008, Welch, Ley et al. 2012, Dovey, Cooper et al. 2017). Experimental studies have further confirmed this model. While *FLT3*-LMs in murine bone marrow transplantation assays as well as in knock in mice only result in myeloproliferative phenotypes (Kelly, Liu et al. 2002, Li, Piloto et al. 2008) leukaemic disease development can be induced by coupling *FLT3*-LMs with other recurrent genetic aberrations like inv(16) (Kim, Kojima et al. 2008), *RUNX1-RUNX1T1* (Schessl, Rawat et al. 2005), *PML-RARA* (Kelly, Kutok et al. 2002), *NPM1* mutations (Mupo, Celani et al. 2013, Dovey, Cooper et al. 2017) or *TET2* mutations (Shih, Jiang et al. 2015). Together this suggests that *FLT3*-LMs in isolation are insufficient to initiate leukaemia and that coupling with specific additional genetic aberration is necessary and sufficient for disease transformation in these models.

Tumour evolution is a further characteristic of AML disease trajectories[9]. This has been recognised since the 1980s when changes in cytogenetic characteristics were observed by longitudinal karyotype evaluation at disease presentation and at disease recurrence. Both increasing complexity as well as loss of cytogenetic aberrancies were observed through individual disease courses

[9] In the Beat AML sample cohort (Tyner, Tognon et al. 2018), studied in paper II, 608 samples from 519 individuals were exome-sequenced. A total of 58 individuals were serially sampled, 44 twice, 12 three times and 2 individuals four times. As a reference regarding the pattern of tumour evolution I provide a supplemental clinical table and a graphically representation of somatic variant dynamics during disease trajectories of these individuals (Supplementary figure 1).

(Garson, Hagemeijer et al. 1989, Kern, Haferlach et al. 2002). By studying temporal molecular patterns of recurrently mutated genes in AML two discrete evolutionary pathways have been suggested: i) persistence of the leukaemic founder clone followed by mutation gain and disease recurrence and ii) progression of minor sub-clones following a branched evolutionary pattern (Ding, Ley et al. 2012, Hirsch, Zhang et al. 2016).

Changes in *FLT3*-LM pattern are frequent during AML disease advancement, and emergence of *FLT3*-LMs in myelodysplastic syndrome is associated with progression to AML (Horiike, Yokota et al. 1997). Relapse in *FLT3*-LM patients is recurrently characterised by loss of the wild type allele though copy neutral loss of heterozygosity (Thiede, Steudel et al. 2002, Stirewalt, Pogosova-Agadjanyan et al. 2014, Loke, Akiki et al. 2015). Gain or loss of *FLT3*-LMs upon disease re-presentation is also not uncommon, and shifts from one to an alternative *FLT3*-LM variant has been described (Nakano, Kiyoi et al. 1999, Hovland, Gjertsen et al. 2002, Kottaridis, Gale et al. 2002, Shih, Huang et al. 2002, Schnittger, Schoch et al. 2004, Tiesmeier, Muller-Tidow et al. 2004, Warren, Luthra et al. 2012, Wakita, Yamaguchi et al. 2013, Garg, Nagata et al. 2015). The relationship between *FLT3*-LM alleles and *FLT3* wild type alleles has additionally been shown to correlate with disease outcome, where both loss of the wild type allele (Whitman, Archer et al. 2001) as well as predomination of the *FLT3*-LM allele is associated with inferior outcome (Thiede, Steudel et al. 2002, Gale, Green et al. 2008). Similar patterns have been described for other somatic variants in AML (Hirsch, Zhang et al. 2016). Although less frequent than in *FLT3*-LM AML, *NPM1* mutation status has been demonstrated to be changeable through disease courses. This was first reported in 2014 when Webersinke et al. reported an AML case study describing a shift in *NPM1* variant between diagnosis and disease recurrence (Webersinke, Kranewitter et al. 2014), and has later been validated to be a recurring finding in larger cohorts, where approximately 9-10% of NPM1 positive AML cases were described as NPM1 wild-type at time of disease recurrence (Hollein, Meggendorfer et al. 2018, Cocciardi, Dolnik et al. 2019).

Cancer stem cell theory

Most multicellular organisms develop from a unicellular origin and gradually expand to the size of up to several trillions of individual cells. An average human body is approximated to comprise around 3.72 x 10^{13} cells (Bianconi, Piovesan et al. 2013), all characterised by approximately the same DNA sequence. Despite this there are up to several hundred morphologically distinct cell types in a typical metazoan body, including the human (Valentine, Collins et al. 1994). This is the result of cell diversification though differentiation. Through epigenetic remodelling, expression of distinct combinations of genes and through post-translational modification of gene products cells with the same DNA sequence can manifest very different phenotypes and properties (Farlik,

Halbritter et al. 2016, Liu, Beyer et al. 2016). These mechanisms not only direct human ontogeny but are crucial in the orientation of adult tissue homeostasis, composed and maintained of cells with varying differential potential and self-renewal capabilities (Biteau, Hochmuth et al. 2011).

Despite mutations occurring in all somatic cells, the ability of a single cell to evolve through somatic evolution is restrained by the replicable properties of the cell. Most propagating human cells are short lived and eventually terminally differentiate and exhaust. This fact suggested the hypothesis of somatic stem cells as the cellular origin of cancer development (Cairns 1975), as well as the concept of stem cell restriction: the number of stem cells and stem cell divisions ultimately confine somatic evolution by natural selection and the probability of complex adaptations (Germain 2012). Recently, tissue specific stem cell division quantification estimates have been demonstrated to correlate with cancer incidence corroborating this hypothesis (Tomasetti and Vogelstein 2015). While the traditional focus of somatic mutation theory has been on germline variants and somatic mutations caused by exposure to mutagens Tomasetti and colleagues postulate that random errors related to DNA replication in stem cells largely explain tissue specific cancer incidence irrespective of environmental conditions (Tomasetti, Li et al. 2017).

In the haematopoietic tissue functional heterogeneity has predominantly been explored by expanding haematopoietic cells *in vitro* and in animal models. It has through such experimental approaches been established that not all haematopoietic cells form colonies and not all haematopoietic cells engraft or can sustain engraftment in immunodeficient mice (Bruce and Van Der Gaag 1963). This has led to a model of the haematopoietic system as an interdependent hierarchy of differentiation processes. The discrete cellular components are related to each other through lines of descent where the cells express discrete properties and functions, including proliferative capabilities and life spans. At the top of the hierarchy the long-lived haematopoietic stem cells reside. These cells are not yet lineage restricted and can provide progeny that can differentiate to any haematopoietic cell type. Descending down the hierarchy lineage commitment increases, and the potential fates of individual cells are gradually restricted while more specialised functional traits are expressed. Haematopoietic stem cells are currently operationally defined as cells capable of reconstituting the entire blood system, coupling self-renewal capacity as well as multi-lineage differential potential. A unifying descriptive delineation of haematopoietic stem cells has been challenging to establish, and the validity of various proposals combining cellular surface proteins as markers remain controversial (Pinho and Frenette 2019).

For a haematopoietic cell to become a source of leukaemia it needs to couple two fundamental and naturally opposing properties: immortalisation and proliferation. As an explanation for this it has been suggested that the orderly organisation of the haematopoietic system is somewhat retained in haematopoietic cancers, where leukaemic stem cells confine the apex of a hierarchy, sustaining the

leukaemia. This claim has been experimentally supported by work presented by John Dick and his associates on AML xenograft models. The first AML engraftment was reported by Lapidot in John Dick´s group in 1994 (Lapidot, Sirard et al. 1994), and in 1997 Bonnet and Dick demonstrated the hierarchical organisation of AML, characterised by cell populations of varying self-renewal capacity (Bonnet and Dick 1997). In analogy with the delineation of normal haematopoietic stem cells leukaemic stem cells are functionally defined by their ability to serially initiate and maintain leukaemia in immunocompromised xenograft models (Hope, Jin et al. 2004, Lane and Gilliland 2010).

The clinical importance of leukaemic stem cells is supported by relationships between high expression of genes associated with leukaemic stem cells and adverse outcome in AML (Gentles, Plevritis et al. 2010, Eppert, Takenaka et al. 2011). Leukaemic stem cell theory emphasises functional multiplicity within the leukaemic cell compartment. Recently the molecular resolution of intra-leukaemic heterogeneity has been increased by the application of next generation sequencing approaches. This has demonstrated that leukaemic samples are characterised by epigenetic and transcriptional diversity, defined as contribution of multiple haematopoietic regulatory programs simultaneously. Single cell profiling has revealed that this diversity derives from two principal sources: i) cells characterised by non-conventional transcriptional states as well as ii) inter-cellular heterogeneity across differentiation spaces (Levine, Simonds et al. 2015, Corces, Buenrostro et al. 2016, van Galen, Hovestadt et al. 2019). Advances in cell-sort technology and improvement in knowledge of cell-identity markers has further enabled the identification and functional characterisation of pre-leukaemic stem-cells within AML specimens, characterised by some, but not all genetic variants present in the corresponding bulk leukaemic sample, as well as the ability to provide multilineage progeny in functional assays (Jan, Snyder et al. 2012, Shlush, Zandi et al. 2014).

Despite *FLT3* being first discovered and described as a haematopoietic stem cell marker (Matthews, Jordan et al. 1991, Small, Levenstein et al. 1994), the literature exploring *FLT3*-LM AML within the conceptual framing for cancer stem cell theory is less elaborated that the body of knowledge of *FLT3* and somatic mutation theory and tumour evolution theory. Samples derived from *FLT3*-LM individuals have been shown to engraft readily in immunocompromised mice, suggesting that at least some *FLT3*-LM AML cells possess leukaemic stem cell properties (Rombouts, Blokland et al. 2000). Levis and colleagues further validated this observation by comparing the *FLT3*-LM allele distribution in primary stem cell enriched and bulk samples demonstrating that *FLT3*-LMs were present within the leukaemic stem-cell compartment (Levis, Murphy et al. 2005). The study of *FLT3*-LM knock in mice has demonstrated numerical as well as functional aberrancies within the haematopoietic stem cell pool. This has been interpreted as proof

of *FLT3*-LM expression as well as functional impact, characterised by haematopoietic stem cell depletion as well as impaired transplant capabilities (Chu, Heiser et al. 2012). Other investigators have reassessed the importance of *FLT3* mediated haematopoietic regulation, demonstrating that both *FLT3* as well as FLT3-ligand are dispensable in the maintenance of murine haematopoiesis, as well as in posttransplant haematopoietic expansion (Buza-Vidas, Cheng et al. 2009). Expression of *FLT3* on long-term haematopoietic stem cells has further been questioned based on functional exploration of various haematopoietic stem cells populations as well as lineage tracing experiments. Both suggest that *FLT3* transcription is associated with the transition to multi-potent progenitor cells and loss of self-renewal capacity (Adolfsson, Borge et al. 2001, Boyer, Beaudin et al. 2012). This claim was recently substantiated by single cell RNA experiments, identifying a strong negative correlation between gene expression of haematopoietic stem cell related genes and *FLT3* mRNA transcripts (Mead, Neo et al. 2017). Functionally it has further been shown that live cells within the blast compartment of *FLT3*-LM mutated AML samples are functionally diverse and characterised by heterogenous activation of STAT3 and STAT5 following *in vitro* stimulation with granulocyte colony stimulating factor (Irish, Hovland et al. 2004). Together this line of findings suggests that the impact of *FLT3*-LMs may vary substantially across phenotypically diverse cells within single leukaemic cell compartments.

Complementary and conflicting models

In addition to somatic mutation theory, tumour evolution theory and cancer stem cell theory I identified a number of publications exploring alternative explanatory models. A handful of publications focused on the preleukaemic stages of AML, presenting compelling evidence of AML as a progression of somatic evolution. Epigenetic contributions in the development of AML were explored in a substantial number of papers, and a few reports investigated the relationship between leukaemic cells and their environment. I shall now review these contributions.

Somatic evolution and genomic diversification

According to evolutionary theory biodiversification is a necessary feature of sustained life in a dynamically changing environment. As mutations can result in altered gene products, both in qualitative and quantitative terms, changes in DNA is a primary source for divergence, expanding the possibility of transcriptional states and corresponding cellular properties. Although genetic recombination is the prevailing source of biodiversification in most metazoans, DNA integrity is continuously broken, resulting in postzygotic somatic diversification (Loewe and Hill 2010, Forsberg, Gisselsson et al. 2017, Risques and Kennedy 2018). Somatic evolution and somatic cells as units of selection represents an opposing force to the multilevel systems involved in the

maintenance of the integrity of the organism. Cancer can in this model be understood in terms of the conflict between evolutionary processes in two dimensions: i) the evolutionary force favouring cohesion of the individual and ii) the evolutionary trajectories of single cells, continuously diversifying and exploring novel trails (Cairns 1975, Merlo, Pepper et al. 2006). Cancer would in this view occur when the second dimension overrides the first.

Mutations are defined as persistent changes in the DNA sequence and results from errors in DNA replication or DNA damage followed by low fidelity repair. Mutations are frequently assumed to be stochastic and agnostic, meaning that the occurrence of a mutation is independent of its translational consequences (Hershberg 2015). Mutations are, however, bio-mechanistic and bio-chemical events and mutation patterns are thus non-random, reflecting specific underlying causes of DNA damage and/or impaired repair mechanisms (Loewe and Hill 2010, Alexandrov, Nik-Zainal et al. 2013, Alexandrov, Ju et al. 2016). The distribution of cancer related somatic mutations has been described as asymmetrical, which has been attributed to patterns of DNA replication timing as well higher order genomic organisation and epigenetic configurations (Woo and Li 2012, Liu, De et al. 2013, Polak, Karlic et al. 2015).

AML has an abrupt clinical presentation and until recently little was known about the dynamics of leukaemogenesis leading up to disease eruption. Exploration of large biobanks of healthy blood cells by capture based deep next generation sequencing methods has resulted in identification of early stages of leukaemogenesis that previously have been unobserved. These studies have demonstrated that acquisition of somatic genetic variants, including mutations in leukaemia associated genes like *DNMT3A*, *ASXL1* and *TET2*, are common events in normal haematopoietic stem and progenitor cells. Such cells have additionally been shown to frequently expand disproportionally with age, resulting in clonal haematopoiesis, which is associated with an increased risk of presenting with a haematological malignancy as well as all-cause mortality (Genovese, Kahler et al. 2014, Xie, Lu et al. 2014, Young, Challen et al. 2016, Acuna-Hidalgo, Sengul et al. 2017, Zink, Stacey et al. 2017, Desai, Mencia-Trinchant et al. 2018). Similar observations have been made across various tissue types, including the oesophagus, the endometrium, and the skin, where mutations in certain cancer related genes have been demonstrated to be prevalent and persisting, including *NOTCH1*, *NRAS*, *KRAS*, *PIK3CA* and *TP53* (Martincorena, Roshan et al. 2015, Martincorena, Fowler et al. 2018, Risques and Kennedy 2018, Suda, Nakaoka et al. 2018). It has furthermore been shown that the number of somatic variants in

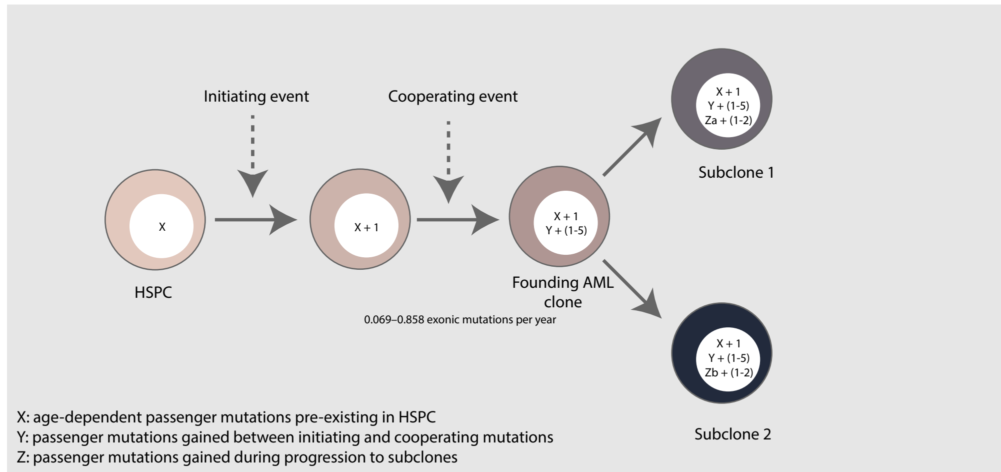


Figure 9: Model of leukaemogenesis incorporating accumulation of random passenger mutations as a function of time. Leukaemogenesis initiates in a haematopoietic stem/progenitor cell that already have accumulated a number of background mutations. Acquisition of a somatic variant conferring selective fitness initiates leukaemogenesis. Over time additional passenger mutations are gained until accumulation of 1-5 cooperating mutations ultimatly transforms the cell to a leukaemic cell. The founding clone continue to diversify giving rise to subclones characterised by additional passenger and driver mutations. Figure adapted from Welch, Ley et al. 2012.

HSPC: Haematopoietic stem/progenitor cell.

healthy haematopoietic stem cells and leukaemic cells is comparable and correlate strongly with chronological age (Welch, Ley et al. 2012, Lo Sardo, Ferguson et al. 2017). Estimations based on the quantification of somatic variants in healthy haematopoietic stem cells suggest that each year throughout life approximately 10 new somatic variants sequentially accumulate in every haematopoietic stem cell, of which only a small fraction are located in protein coding regions. The yearly incidence rate of exonic mutations is estimated to be as low as 0.13 +/- 0.02 per haematopoietic stem cell (Welch, Ley et al. 2012). This suggests that mutations accumulate over time, and that most somatic variants are not causally involved in leukaemogenesis. Even distribution of variants across different regions of the genome (e.g. protein coding sequence, regulatory sequence and non-regulatory sequence) and a recurring mutational pattern characterising both healthy as well as AML exomes, (both dominated by C>T/A>G transitions, probably resulting from deamination of methylcytosine residues leading to subsequent transitions) further support random accumulation of somatic variants as a function of time (Welch, Ley et al. 2012) (Figure 9).

Continuous acquisition and persistence of mutations suggest that metazoans are characterised by increasing genomic complexity as a function of time. An analogous pattern of gradual genomic diversification is increasingly recognised as a part of cancer development. Metaphase karyotyping has revealed cytogenetic diversity in 15-20% of AML cases (Bochtler, Stolzel et al. 2013, Baron, Stevens-Kroef et al. 2018). Inferred cell population size by variant allele fraction patterns of

samples analysed by bulk next generations sequencing assays has suggested that intra-leukaemic genomic heterogeneity is common (Welch, Ley et al. 2012, Cancer Genome Atlas Research Network 2013). Single cell sequencing techniques have shed light on the profoundness of genomic diversity and confirmed that bulk AML samples frequently consist of numerous genotypically diverse cell populations (Paguirigan, Smith et al. 2015, Potter, Miraki-Moud et al. 2018, van Galen, Hovestadt et al. 2019). Engraftment studies have further supported clonal complexity by demonstration of genetic variation within the leukaemic stem cell compartment (Klco, Spencer et al. 2014, Vick, Rothenberg et al. 2015, Shlush, Mitchell et al. 2017, Wang, Sanchez-Martin et al. 2017). Such heterogeneity is well described in *FLT3*-LM AML where *FLT3*-LMs frequently characterise only a minor fraction of cells in leukaemic specimens, and not infrequently multiple *FLT3*-LMs are identified (Horiike, Yokota et al. 1997, Kottaridis, Gale et al. 2001, Gale, Green et al. 2008, Meshinchi, Stirewalt et al. 2008, Borthakur, Kantarjian et al. 2012, Schranz, Hubmann et al. 2018, Blatte, Schmalbrock et al. 2019). Moreover, treatment with FLT3 inhibitors is characterised by rapid emergence of non-*FLT3*-LM cell populations characterised by mutations in alternate signalling genes, including *NRAS*, *PTPN11* and *CBL*, suggesting that AML is a genetically composite disease (McMahon, Ferng et al. 2019, Zhang, Savage et al. 2019).

Epigenetic diversification

Cell fate disruption has long been recognised as a core feature of AML. Block in terminal differentiation is a characteristic feature of leukaemic cells, and lineage-specific transcription factors are recurrently mutated or involved in structural rearrangements (Tenen 2003). The identification of several recurrently mutated genes coding for critical epigenetic regulators, including DNA methylation-related genes, chromatin modifiers as well as cohesin-complex genes (Cancer Genome Atlas Research Network 2013), led to increased attention to epigenetic dysregulation as a contributor of leukaemogenesis. However, the explanatory power of epigenetic contributions in oncogenesis exceeds the translational effects of mutated genes. Epigenetics is a collective term referring to molecular features that i) contribute in the instruction of gene expression and ii) are vertically passed down to cellular progeny through mitotic and meiotic inheritance, much like the transfer of information through DNA replication (Berger, Kouzarides et al. 2009, Cavalli and Heard 2019). This has led to the postulation that epigenetic “hits” are complementary causal contributions in leukaemogenesis analogous to the acquisition of somatic variants (Figure 10). Based on the study of DNA methylation patterns in myelodysplastic syndromes and AML, with a particular focus on disease progression, Jiang and colleagues suggest that aberrant DNA methylation is a prevailing mechanism for tumour suppressor silencing, surpassing the effect of typical loss of function mutations (Jiang, Dunbar et al. 2009).

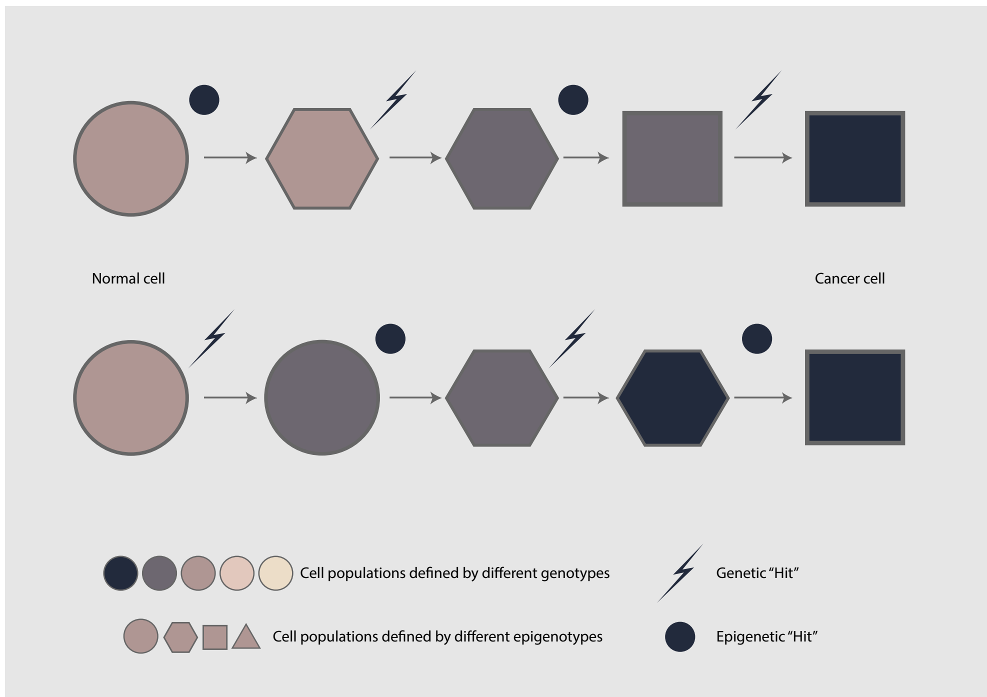


Figure 10: Illustration of tumour evolution where accumulation of "hits" include epigenetic aberrancies. Various sequences of genetic and epigenetic "mutations" contribute to the transformation of normal cells to cancer cells.

Epigenetic contribution in leukaemogenesis is supported by observations from the study of *FLT3*-LM AML. *FLT3*-LMs frequently co-occur with mutations in genes involved in epigenetic regulation, including *DNMT3A* and *TET2* (Cancer Genome Atlas Research Network 2013, Garg, Nagata et al. 2015, Tyner, Tognon et al. 2018). *DNMT3A* is proposed to behave as a tumour suppressor in haematopoietic cells, and loss of *DNMT3A* expression is associated with enhancement of haematopoietic stem self-renewal and impaired differentiation through hypomethylation of important regulatory enhancer regions. When combined with *FLT3*-LMs acute leukaemia rapidly manifests in animal models (Yang, Rodriguez et al. 2016). In a mouse model *FLT3*-LMs in combination with a *TET2* loss of function mutation resulted in epigenetic silencing of *GATA2*, an event suggested to be involved in leukaemic transformation (Shih, Jiang et al. 2015). Similarly, epigenetic deregulation of *GATA1* has been proposed to be involved in leukaemic transformation in *NPM1* and *FLT3*-LM AML (Sportoletti, Celani et al. 2019). Regulatory RNA has also been implicated in leukaemogenesis. An example is *MicroRNA 155* that has been shown to be up-regulated in *FLT3*-LM AML (Garzon, Garofalo et al. 2008, Whitman, Maharry et al. 2010). Experimentally it has been shown than induction of *MicroRNA 155* is a down-stream signalling effect of FLT3, which ultimately results in dysregulation of the myeloid transcription factor *PU.1* (Gerloff, Grundler et al. 2014). Comparing *FLT3*-LM positive and negative primary

leukaemic samples Cauchy and colleagues further identified a chromatin signature specifically associated with *FLT3*-LM signalling, shaped by activation of STAT and MAPK pathways resulting in specific changes in the regulatory conformation of the genome (Cauchy, James et al. 2015).

Cell identity and the value of single gene-variants

Somatic mutations may result in phenotypic change upon translation, and occasionally the phenotypic change has an effect on cellular fitness. Several publications presented data that underscored the importance of gene-context as a variable that influences the value of somatic variants. These findings suggest that identical mutations and subsequent gene products may confer variable qualities dependent on individual context and connectivity (Schaefer and Serrano 2016).

FLT3-LMs display remarkable tissue and cell-of-origin specificity. Expansion of cells with somatic changes in the *FLT3* gene have almost exclusively been described in cancers originating from cell-subsets known to express FLT3 (Yokota, Kiyoi et al. 1997, Serve, Flesch et al. 1999). This suggest that the oncogenic effect of *FLT3*-LMs is restricted to features of the transcriptome: a *FLT3*-LM appears to provide a significant change in cellular fitness only when transcribed. The relationship between *FLT3*-LMs and cell ontogeny and cell identity has been experimentally substantiated. By comparing the effect of *FLT3*-LMs in adult and foetal murine haematopoietic progenitor cells Porter and colleagues demonstrated that the ability of *FLT3*-LMs to induce self-renewal as well as myeloid commitment programs in part was dependent on the preceding transcriptional state of the cell (Porter, Cluster et al. 2016), suggesting that the size and direction of fitness change introduced by *FLT3*-LMs is context dependent.

Identification of some but not all leukaemia associated mutations in otherwise healthy individuals (Genovese, Kahler et al. 2014, Xie, Lu et al. 2014, Young, Challen et al. 2016, Acuna-Hidalgo, Sengul et al. 2017, Zink, Stacey et al. 2017, Desai, Mencia-Trinchant et al. 2018), as well as persistence of the same mutations post induction therapy and in enduring remissions (Hirsch, Tang et al. 2017, Jongen-Lavrencic, Grob et al. 2018) suggest that the sequential order of mutation acquisition influence cellular phenotype and the probability of successive expansion. Recurring variant allele patterns suggest that *FLT3*-LMs are acquired late in the leukaemogenic process, as they almost always co-exist with somatic variants with higher variant allele frequencies (Malkin 2011). This interpretation has been supported by single cell sequencing studies demonstrating that *FLT3*-LMs frequently characterise only a subset of leukaemic cell populations (Shouval, Shlush et al. 2014, Potter, Miraki-Moud et al. 2018). Experimentally it has been demonstrated that when *FLT3*-LMs are introduced in “healthy” haematopoietic stem cells the ability of self-renewal is disrupted, resulting in depletion of the haematopoietic stem cells pool (Chu, Heiser et al. 2012). Similarly, introduction of *FLT3*-LMs in cells that already manifest an established leukaemic

phenotype result in synthetic lethality (Oveland, Wergeland et al. 2012). This is further supported by the context dependent prognostic association related to *FLT3*-LMs, influenced by co-occurrence of mutations in alternate genes (Papaemmanuil, Gerstung et al. 2016). In sum this implies that the preceding genotype may influence the translational effects of somatic variants.

These principles are not restricted to *FLT3*-LM AML. In myeloproliferative neoplasms it has been demonstrated that the order of which genes are mutated influence both cellular characteristics, as well as clinical features and response to targeted therapy (Ortmann, Kent et al. 2015). The cell of origin influences the oncogenic effects of *MLL*-rearrangements in AML (Krivtsov, Figueroa et al. 2013). Chao and colleagues have further elaborated on this principle. They were able to de-differentiate primary *MLL*-rearranged AML blasts to induced pluripotent stem cells and re-differentiate the cells into various non-haematopoietic cell-types, all presenting normal phenotypes. When cell-identity was redirected towards haematopoietic destinations leukaemic properties re-emerged (Chao, Gentles et al. 2017). Analogously a much older experiment demonstrated that transduction of a leukaemogenic *MLL* fusion gene in haematopoietic progenitor cells of various differentiation stages recurrently resulted in maturation arrest at the same differentiation level (Cozzio, Passegue et al. 2003). Observations from germline variants and hereditary cancer predisposition syndromes provide theoretical support. It is well recognised that germline genetic variants frequently result in composite phenotypes, suggesting substantial molecular variability upon gene-product variant integration in various gene-environments. Furthermore, cancer penetrance is variable and frequently tissue specific. Li-Fraumeni syndrome, characterised by *TP53* germline variants, is known to increased risk of leukaemic presentation (Malkin 2011). The lifetime risk of leukaemia is, however, far from comparable to the reported transformation rate in somatically *TP53* mutated clonal haematopoiesis where a 100% transformation rate recently was reported by Desai et al. (Desai, Mencia-Trinchant et al. 2018). Conversely, in myeloproliferative neoplasms is has been shown that *TP53* mutated subpopulations of cells are frequently present but only occasionally expand or transform to AML (Kubesova, Pavlova et al. 2018).

Cell extrinsic contributions in adaptation, selection and convergence

The literature selected for the dissertation synthesis also included studies that suggest that the relevant gene-environment exceeds the limits of individual cells. This perspective was represented primarily through reports related to cell- and immunotherapy, but also comprised a handful of papers presenting evidence of context dependency with regards to therapeutic responses, as well as reports of evolutionary trajectories influenced by externally enforced selection pressure (Smith, Wang et al. 2012, Landau, Carter et al. 2014).

It is estimated that the daily haematopoietic output of terminally differentiated haematopoietic cells approximates the magnitude of 5 $x10^{11}$. The yield has been theorised to have a 10-20 folds expansive potential under conditions of demand (Creutzig, Zimmermann et al. 2016, Bolouri, Farrar et al. 2018). Maintenance of homeostatic conditions requires relentless production and expansion of cells from the top of the hierarchy to replenish the cells that have expired or been lost. Establishment of individual cell identity within the hierarchy is a co-produced process involving environmental cues (Zhu, Adli et al. 2013). The balance is sustained and stabilised through multiple systemic mechanisms, including circadian (Golan, Kumari et al. 2018), metabolic (Agathocleous, Meacham et al. 2017), endocrine (Abdelbaset-Ismail, Suszynska et al. 2016), immunological (Baldridge, King et al. 2010) and inflammatory pathways (Wilson, Laurenti et al. 2008). Cell extrinsic signals are internalised and transformed to altered cell behaviour through intra-cellular signalling pathways that ultimately result in the activation or inactivation of transcription factors responsible for the regulation of gene expression. Recent reports further suggest that activation of intra-cellular signalling pathways directly modify chromatin structure and histone configuration through post-translational modifications, directly contributing in epigenetic remodelling (Cavalli and Heard 2019). What follows is that similar environments result in phenotypic convergence across multiple cellular entities with a similar genetic and epigenetic configuration (Stadhouders, Filion et al. 2019). In line with this haematopoietic stem cells located at different organ sites differ with respect to differentiation trajectories and properties (Benz, Copley et al. 2012). Functional differences among haematopoietic stem cells in the same niche have been attributed to variation in precise location and relation to cellular and non-cellular niche components, like osteoblast, vascular cells and the stroma (Ghobrial, Detappe et al. 2018).

The emphasis of microenvironmental and systemic contributions with regards to cell-identity adds to the theories of tumour evolution theory that cellular competition, adaptation and selection occurs in an adaptive and dynamically changing cell extrinsic environment. Evidence suggests that such variation is relevant with regards to leukaemic cell properties. It has been shown that culture conditions influence anti-leukaemic responses to a variety of therapeutic compounds (Karjalainen, Pemovska et al. 2017). *FLT3*-LM cells have been demonstrated to be less sensitive to direct FLT3 inhibition when grown in culture media supplemented with FLT3-ligand, cytokines normally produced in the bone marrow, or when grown in co-culture with bone marrow derived stromal cells (Sato, Yang et al. 2011, Yang, Sexauer et al. 2014, Sung, Sugita et al. 2019). This suggest that leukaemic cells retain a certain level of connectivity and responsiveness with regards to their surroundings, resulting in cell-extrinsic environment dependency with regards to gene variant properties and dependencies.

Changes in gene environment occasionally result in emergence of cell populations with shared genetic or phenotypic features. Emergence of clonal haematopoiesis following medical cancer treatment is not uncommon (Coombs, Zehir et al. 2017). Wong and colleagues showed that discrete therapeutic strategies resulted in expansion of genetically defined cellular subsets, characterised by DNA damage response genes following chemotherapy and *DNMT3A* mutations succeeding haematopoietic stem cell transplantation, respectively (Wong, Miller et al. 2018). Molecular characteristics of treatment related AML similarly reflect preceding therapy. Del(5q) and/or partial or complete deletion of chromosome 7, *TP53* mutations and chromosome 17 alterations are associated with previous exposure to alkylating agents. Similarly, *MLL*- or *RUNX1*-rearrangements are linked to preceding topoisomerase II inhibitor treatment (McNerney, Godley et al. 2017). Furthermore, the choice of anticancer treatment appears to have an effect on subsequent molecular disease progression patterns. Disease recurrence following chemotherapy has been shown to converge towards a more stem-cell-like state, as defined by genome-wide DNA methylation profiling (Triche Jr, Johnson et al. 2018). Post-transplant disease re-presentation has analogously been characterised by deregulation of gene expression in a pattern hypothesised to promote immune escape from T-cell mediated allorecognition (Christopher, Petti et al. 2018).

In *FLT3*-LM AML treatment failure of FLT3 directed tyrosine kinase inhibitors after initial response is characterised by emergence of up to several novel *FLT3* mutations conferring resistance (Man, Fung et al. 2012, Smith, Wang et al. 2012, Baker, Zimmerman et al. 2013, Smith, Paguirigan et al. 2017), or as previously mentioned cell populations characterised by alternate mutations in signalling genes (McMahon, Ferng et al. 2019, Zhang, Savage et al. 2019). Analogously *IDH1* and *IDH2* isoform switching has been observed in relation to IDH-targeted therapy (Harding, Lowery et al. 2018, Intlekofer, Shih et al. 2018).

Converging evolution is further a feature described in relation to cancer cell migration and metastasis, where cancer-cells from various primary sites typically converge upon establishment in the same distant organ location (Cunningham, Brown et al. 2015). This illustrates the orientating potency of environmental conditions and suggests that endogen variation in gene-environments may influence disease trajectories.

The observations presented above suggest that variation in mutational profiles in part can be accounted for by variation in individual cell heritage as well as supra-cellular gene environments. Of particular interest with regards to the relevance of such mechanisms are patterns of phenotypic and molecular convergence within cancers: plurality of mutations in the same gene or genes with similar functions, that occur independently in various cells at various sites and at various time points within the same tumour. This observation is frequently attributed to Gerlinger and colleagues who in 2012 demonstrated molecular convergence through multi-region sequencing of renal

carcinomas (Gerlinger, Rowan et al. 2012). Numerical variation of *FLT3*-LM alleles within single individuals is as previously mentioned, however, a recurrent finding in the AML research literature, first described already in 1997, and later repeatedly reproduced (Horiike, Yokota et al. 1997, Kottaridis, Gale et al. 2001, Gale, Green et al. 2008, Meshinchi, Stirewalt et al. 2008, Borthakur, Kantarjian et al. 2012, Schranz, Hubmann et al. 2018, Blatte, Schmalbrock et al. 2019). Convergent phenotypic and molecular evolution is also observed in other subgroups of AML. Jang et al. identified more than one *cKIT* mutation in 30 of 69 *cKIT* positive core-binding factor leukaemia specimens (Jang, Yoon et al. 2016), and plurality of *cKIT* mutations have been described to either co-occur in the same cell or to define discrete cell populations (Tan, Liu et al. 2018). Similarly, Itzykson and colleagues described clonal interference between cell populations defined by discrete signalling drivers in 28% of core-binding factor leukaemia (Itzykson, Duployez et al. 2018). Likewise, dual *IDH1* and *IDH2* mutations have recently been described in AML (Platt, Fathi et al. 2015).

Convergent molecular evolution has also been shown across other cancers, including *BTG1* deletions in acute lymphoblastic leukaemia (Waanders, Scheijen et al. 2012), plural *BRAF* and *KRAS* mutations in chronic lymphocytic leukaemia (Vendramini, Bomben et al. 2019), *BRAF* mutations in malignant melanoma (Valachis and Ullenhag 2017), *CTNNB1* mutations in hepatocellular carcinoma (Friemel, Rechsteiner et al. 2015), *CIC*, *FUBP1* and *NOTCH1* mutations in gliomas (Suzuki, Aoki et al. 2015), *PTEN*, *TP53* variants as well as deletion of coding exons of *RUNX1* and amplification of *FGFR2* in breast cancer (Yates, Gerstung et al. 2015), and *KRAS* and *EGFR* mutations in lung cancer (Kalikaki, Koutsopoulos et al. 2008, Marchetti, Milella et al. 2009). Even combinations of mutations have been described to evolve in parallel, like the combination *TP53* and *ATRX* mutations in a patient with glioma (Suzuki, Aoki et al. 2015). Convergent molecular evolution has also been described in premalignant lesions like intra-ductal papillary mucinous neoplasms (Kuboki, Fischer et al. 2019).

Supra-cellular dimensions and causality

The extension of gene-context to consider cell-extrinsic gene-relationships proposes that supra-cellular processes and events influence the onset and transitions related to leukaemogenesis (Ghobrial, Detappe et al. 2018). This is supported by evidence of leukaemic development in animal models characterised by bone progenitor cell dysfunction (Raaijmakers, Mukherjee et al. 2010) and abnormal osteoblast function (Kode, Manavalan et al. 2014). Analogously, cell extrinsic

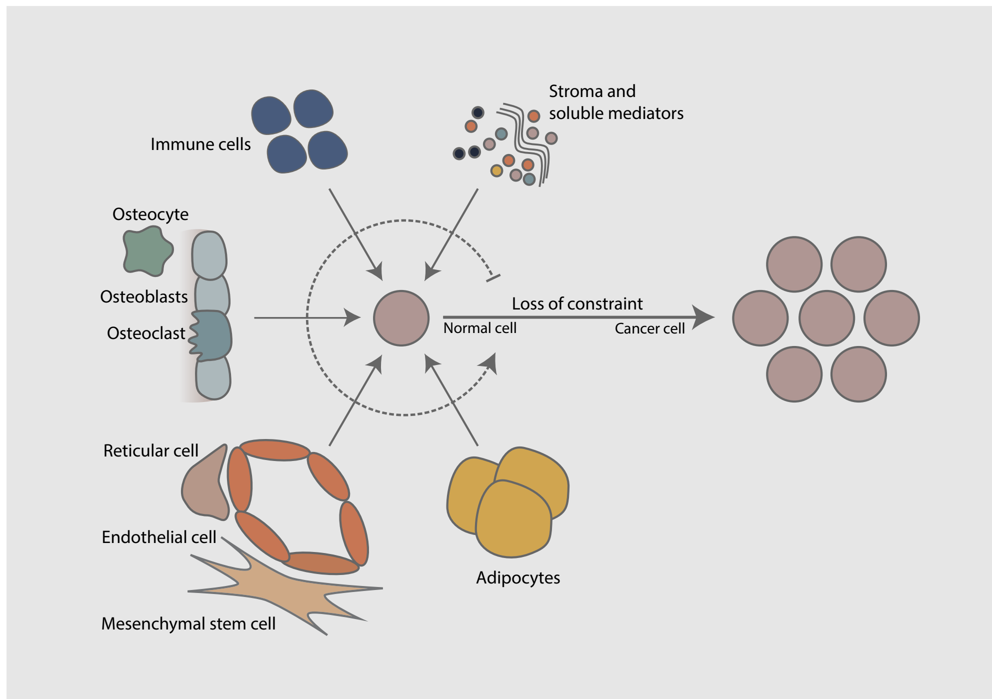


Figure 11: Haematopoietic stem and progenitor cells predominantly reside within the bone marrow. The bone marrow microenvironment is rich and diverse and is frequently subdivided into sub-compartments: vascular niche, the reticular niche and the endosteal niche. Together the various cellular and non-cellular bone marrow components contribute in the orchestration of haematopoietic homeostasis, stress responses and in haematological malignancies. According to the premises of tissue organisation field theory emergence of leukaemia can be explained by bone marrow microenvironment disorganisation resulting in loss of constraint of haematopoietic stem and progenitor cells proliferation.

mechanisms have been implied in human leukaemogenesis in relation to germline variants and their contribution in the development of myeloid malignancies[10] (Dong, Yu et al. 2016).

The immune system is another systemic dimension attributed importance with regards to leukaemia. Allogeneic stem cell therapy is an important treatment modality in high risk AML (Knorr, Goldberg et al. 2019). Comparison of variable graft composition have demonstrated a correlation between the fraction of mature immune cell subsets and relapse rates, suggesting an immune mediated graft versus leukaemia effect (Horowitz, Gale et al. 1990) and a relationship between immunocompetency and leukaemia.

These results in part align with the conceptual framework of tissue organisation field theory presented by Sonnenschein and Soto in 2000 (Figure 11). This theory emerged in response to what

[10] PTPN11 activating mutations, characterizing approximately 50% of patients with Noonan syndrome, are associated with increased risk presentation with a myeloproliferative neoplasm. Mechanistically disease progression has been shown to involve excessive production of the chemokine CCL3 by bone marrow mesenchymal cells (Dong, Yu et al. 2016).

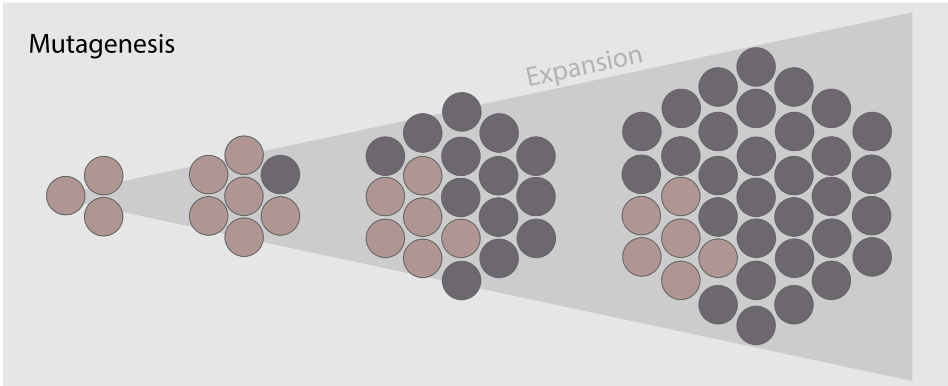
Sonnenschein and Soto interpreted as conceptual inconsistences related to the basic premises of somatic mutation theory. They questioned the assumption of quiescent as the default mode of somatic cells, and argued that expansion is a more fundamental feature, in line with the behaviour of unicellular organisms as well as embryonic stem cells. They further argued that cancer results from the breakdown of biological organisation, hence proposing that cancer is an emergent phenomenon that cannot be explained at a cellular level (Sonnenschein and Soto 2000).

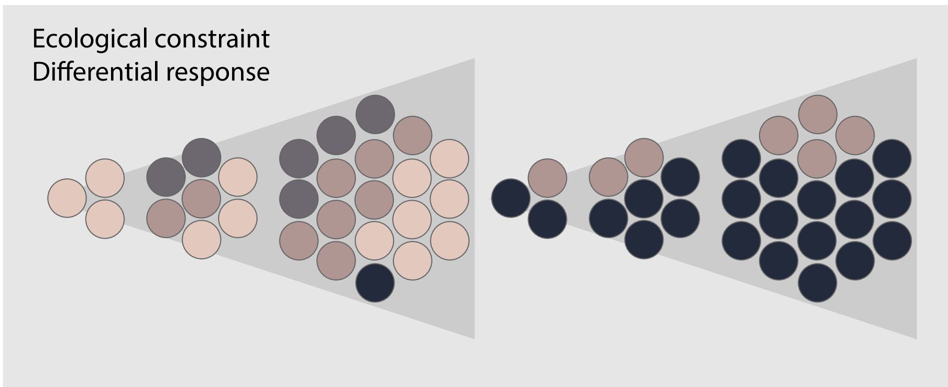
To my knowledge the tissue organisation field theory is not clearly articulated in the *FLT3*-LM AML literature. There are, however, numerous findings that corroborate relationships between features of the cell-extrinsic environment and the relative distribution of *FLT3*-LMs in AML. Positive *FLT3*-LM status has repeatedly been associated with patient features like younger age and female gender (Schnittger, Schoch et al. 2002, Blau, Berenstein et al. 2013). According to somatic mutation theory the age-related incidence increase of AML can be accounted for by acquisition and accumulation of mutations as rate limiting events with regards to disease eruption. This model does, however, not account for discrete age-related differences with regards to specifically mutated genes, as has recently been described not only for *FLT3* but also multiple other gene variants (Creutzig, Zimmermann et al. 2016, Bolouri, Farrar et al. 2018). While the male excess of AML has been attributed to sex-related variation in exposure to mutagens (Cartwright, Gurney et al. 2002), this does not fully account for a female majority among individuals with *FLT3*-LM AML. Recent lines of evidence emphasise age-related changes in the bone marrow tissue composition. Increasing age is associated with gradual corrosion of haematopoietic cohesion resulting in dysregulation of haematopoietic stem cells (Lee, Jeong et al. 2019, Verovskaya, Dellorusso et al. 2019). Sex further shape intra-cellular, micro-environmental and systemic gene-context with temporal variation. In an age dependent manner puberty (Hero, Wickman et al. 2005, Peng, Yu et al. 2018), sex steroid cyclicity (Chaireti, Lindahl et al. 2016, Kempe, Eklund et al. 2018), pregnancy (Nakada, Oguro et al. 2014, Aghaeepour, Ganio et al. 2017) and menopause (Heo, Chen et al. 2015) all influence haematopoietic and immune regulation as well as stromal bone marrow conditions, including the functional properties of bone marrow derived mesenchymal stromal cells (Siegel, Kluba et al. 2013) and bone metabolisme (Khosla and Monroe 2018).

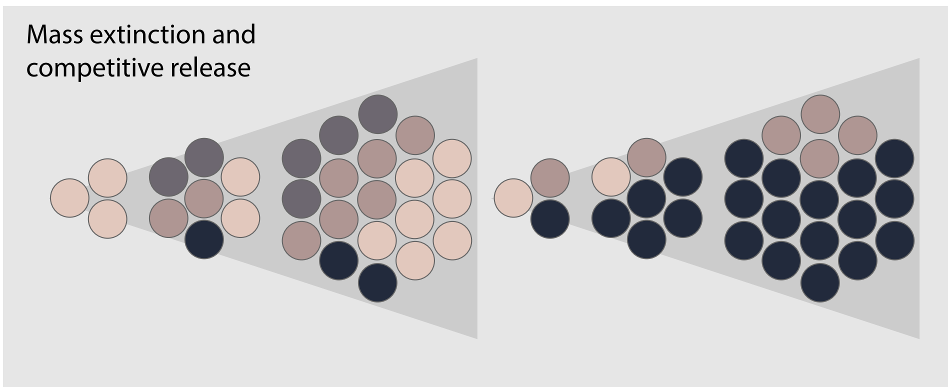
Résumé

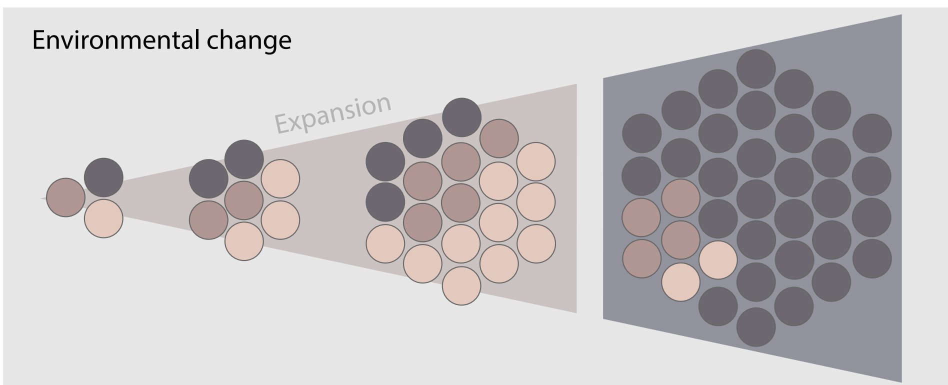
In this section, I have reviewed the research literature on AML (or more precisely, a selection of highly cited research papers studying AML) and discussed it in terms of how it expresses, or lends, support to the various theoretical frameworks for cancer, including somatic mutation theory, tumour evolution theory and cancer stem cell theory. I have also presented and discussed various findings that appear to go beyond these three frameworks and pointed to a variety of complementary models. So far in the discussion, we may conclude that AML is not only a highly heterogenous and dynamic phenomenon but also one that only with difficulty can be accounted for by a single explanatory model. In particular, somatic mutation theory alone is not sufficient to explain the diversity of empirical findings. Indeed, the observations and experimental results presented in this section appear to support explanatory models in which individual cell trajectories are profoundly relational and where phenotypes of course are constrained by genotype but, to the extent that they become persistent, also lend themselves to explanation in terms of their external environment (Figure 12).

Figure 12. Four models of how changes in the relationship between genes and their environment may promote asymmetric changes in cell population trajectories. 1. Mutations may result in increased fitness resulting in relative expansion compare to non-mutated cells. 2. Ecological constrain like targeted treatment or chemotherapy can profoundly alter cell population relationships by selectively targeting or not targeting some cell populations. One example would be targeting FLT3-ITD expressing cells resulting in a fitness advantage for cells not expressing this target. 3. Less specific ecological constraint like autoimmune reactions or reactive bone marrow suppression in response to infection resulting in mass extinction and competitive release. 4. Change in environment that result in alter gene variant fitness. Figure adapted and expanded on from Landau, Carter et al. 2014.


Mutagenesis
Expansion


Ecological constraint
Differential response


Mass extinction and
competitive release


Environmental change
Expansion

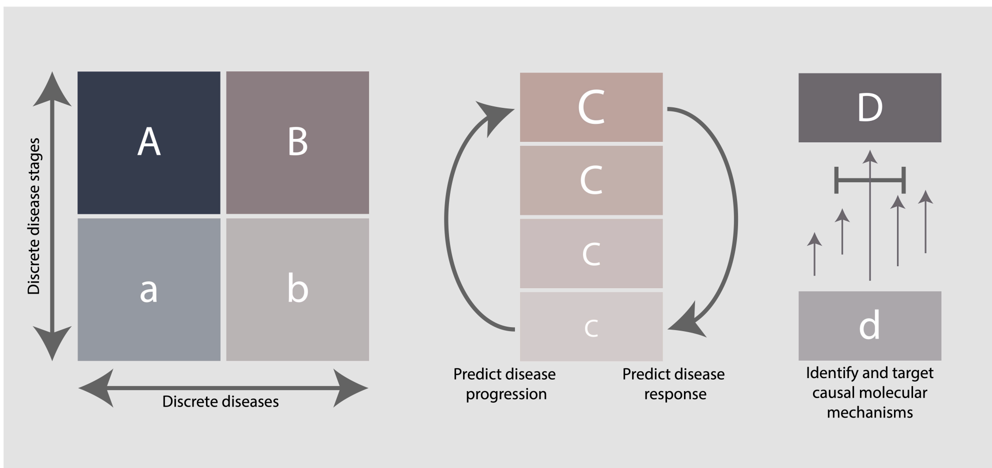


Figure 13: Precision haemato-oncology aims at improving disease management and outcome by i) identification of molecular defined disease boundaries, ii) determination of molecular delineations between disease subgroups, iii) development of molecular tools applicable for risk-adapted clinical management, and iv) identification and exploration of novel druggable molecular targets.

Precise management of AML

Assessment of the literature selection with regards to precision medicine related approaches revealed that the majority of papers explicitly articulated a potential future clinical relevance with regards to their findings. Based on current definitions of precision medicine (Yates, Seoane et al. 2018) close to all applications proposed were precision medicine related. A synthesis of the objectives suggests that a major goal in haemato-oncology is to improve disease specific outcome through a collection of molecular oriented approaches, comprising i) identification of precise disease boundaries, ii) delineation of biologically diverse cases into molecular homogenous disease subgroups, iii) development of molecular tools applicable for risk-adapted clinical management, and iv) identification and exploration of novel druggable molecular targets (Figure 13). In the following I summarise the strategies identified in the literature selection, and I explore how they relate to the theories of leukaemia presented in the previous chapter (Figure 14).

Disease delineation

While identification of certain chromosomal rearrangements[11] is sufficient to confirm AML diagnosis irrespective of blast percentage (Vardiman, Harris et al. 2002, Vardiman, Thiele et al. 2009, Arber, Orazi et al. 2016) most AML cases are diagnosed in accordance with an arbitrary morphological cut-off based on enumeration of blasts in a bone marrow smear (Arber, Orazi et al.

[11] Identification of t(8;21)(q22;q22), inv(16)(p13q22), t(16;16)(p13;q22) or t(15;17)(q22;q12) is sufficient for AML diagnosis, irrespective of blast counts.

2016). The validity of the current cytogenetic boundaries are moreover dubious as these rearrangements are frequently found in the blood of otherwise healthy individuals at low frequencies (Janz, Potter et al. 2003, Song, Mercer et al. 2011), as well as in long term remissions (Miyamoto, Nagafuji et al. 1996, Tobal and Liu Yin 1998). This suggests that these borders are only useful and valid when paired with signs of bone marrow disease involvement. In the literature focusing on expanding the knowledge base with regards to tumour evolution a recurrent argument identified was that sharper diagnostic boundaries could facilitate improved clinical management of AML. More precise disease boundaries could aid earlier disease detection as well as improved disease monitoring and therapy response assessment (Walter, Shen et al. 2012, Corces-Zimmerman, Hong et al. 2014, Genovese, Kahler et al. 2014, Shlush, Zandi et al. 2014). The distribution of causal narratives in the AML literature suggested that acquisition and accumulation of somatic mutations are understood as the prevailing causal contributors and rate limiting events in AML pathogenesis. According to somatic mutation theory molecular boundaries (e.g. mutations or epigenetic hits) separating non-leukaemia and leukaemia do exist. In line with this I found that identification of molecular features causally related to leukaemic transformation and disease progression is a key precision oncology related goal.

Disease stratification

The last decades have been characterised by substantial shifts in the boundaries and sub-categories of AML, resulting in gradual stratification of the disease (Harris, Jaffe et al. 2000, Vardiman, Thiele et al. 2009, Arber, Orazi et al. 2016). The current World Health Organisation (WHO) classification framework for myeloid neoplasms and acute leukaemia (Supplementary table 2) comprises cytogenetic, molecular genetics, cytomorphology, germline variant associated, and aetiology founded sub-groups (Arber, Orazi et al. 2016). According to somatic mutation theory and tumour evolution theory the extensive molecular heterogeneity of AML is suggestive of plural molecular causal chains leading to disease eruption. In accordance with this interpretation I found that delineation of subgroups with shared molecular pathophysiology is an important aspiration in precision haemato-oncology. In relation to a proposed purely genomic classification system of AML the authors argued: “Cancer develops from somatically acquired driver mutations, which account for the myriad biologic and clinical complexities of the disease. A classification of cancers that is based on causality is likely to be durable, reproducible, and clinically relevant” (Papaemmanuil, Gerstung et al. 2016), underscoring the relevance of somatic mutation theory in the evolution of AML disease stratification frameworks.

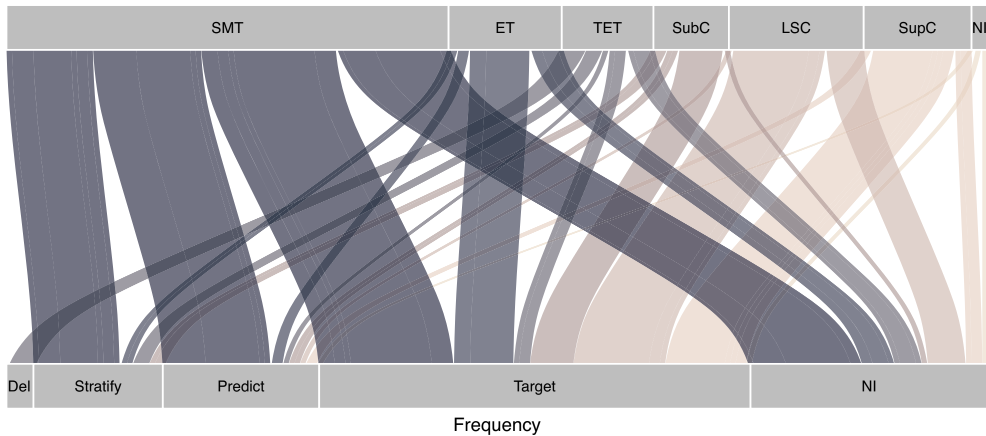


Figure 14: A flow diagram (alluvial plot) visualising the relationship between the various identified theoretical frameworks and precision medicine related approaches. The block sizes represent relative frequency and the stream fields represent how various causal narratives relate to precision medicine related approaches within single papers. In cases where more than one narrative was present I categorised them according to the framework at the highest biological level (SubC>LSC>SubC>TET>ET>SMT). Review articles, reports from clinical trials, meta-analysis and epidemiological reports were excluded from the visual analysis leaving a total of 182 papers.

SMT: somatic mutation theory, ET: epigenetic theory, TET: tumour evolution theory, SubC: subcellular explanations, LSC: leukaemic stem cell theory, SupC: supracellular explanation, NI: not identified, Del: delineation

Predictions and risk-adapted management

The importance of more precise delineation and increased sub-stratification of AML is motivated by aspirations of improving disease specific outcome. Due to the aggressive nature of AML improvements in therapeutic strategies are considered instrumental in achieving such progress (Dohner, Estey et al. 2017). In AML treatment considerations are influenced by factors like availability of treatment, affordability, patient preferences and patient features like age, comorbidities and performance status. Increasingly, however, disease specific characteristics inform clinical decision making, including cytogenetic and molecular features (Cheson, Cassileth et al. 1990, Cheson, Bennett et al. 2003, Dohner, Estey et al. 2010, Dohner, Estey et al. 2017, O'Donnell, Tallman et al. 2017). Disease management plans are guided by assessment and balancing of risk related to probability of obtaining curation or disease control as opposed to the hazard of treatment induced morbidity and mortality (Dohner, Estey et al. 2017). Accordingly, risk classification systems are important clinical tools in the management of AML. Increasing number of strata characterise the development of such risk stratification systems over time (Cheson, Cassileth et al. 1990, Cheson, Bennett et al. 2003, Dohner, Estey et al. 2010, Dohner, Estey et al. 2017, O'Donnell, Tallman et al. 2017). Knowledge generation with regards to risk assessment was strongly represented in the literature, suggesting that predictive refinement is considered an important precision medicine related objective. While the most recent guidelines commend risk

stratification of newly diagnosed AML patients based on characterisation of a selection of cytogenetic and molecular genetic markers [12] and to adapt treatment plans accordingly (Supplementary table 3) (Dohner, Estey et al. 2017, O'Donnell, Tallman et al. 2017), the literature assessment demonstrated plurality of approaches. Expression of single genes, gene-expression profiles, epigenetic features and cell identity quantification were all shown to correlate with outcome in AML. Increasingly also longitudinal risk assessment is recommended. This is primarily obtained by response evaluation following induction therapy and further refines the treatment algorithm, informing post-remission strategies (Dohner, Estey et al. 2017). In sum this suggests that predictive practices are less stringently related to somatic mutation theory than disease delineation and sub-classification.

Targeted therapy

Identification of novel treatment targets was the dominating precision medicine related approach identified in the literature selection. An appraisal of the targeting strategies suggested that efforts aimed at leveraging molecular pathways implicated in disease pathogenesis has dominated the drug development landscape in AML over the last 20 years. The majority of papers focused on inhibition of oncogenic effectors attributed to driver mutations (Ley, Mardis et al. 2008), including FLT3, IDH1 and IDH2 targeted strategies. Analogously, work focused on characterisation of epigenetic mechanisms proposed epigenetic targets or strategies (Jiang, Dunbar et al. 2009), while projects exploring the relationship between leukaemic cells and the microenvironment suggested therapeutic inhibition of leukaemic cell dependencies (Zeng, Shi et al. 2009). Authors exploring temporal disease dynamics offered various rationales for the superior benefit of targeting early (Corces-Zimmerman, Hong et al. 2014) or late (Smith, Wang et al. 2012) somatic variants, respectively. Work characterising various cells sub-sets related to treatment failure, including pre-leukaemic cells and leukaemic stem cells, reasoned that identification and targeting of cell specific features would improve treatment outcomes (Jin, Hope et al. 2006, Somervaille and Cleary 2006, Majeti, Becker et al. 2009, Jan, Snyder et al. 2012, Corces-Zimmerman, Hong et al. 2014). In a subset of papers assumptions with regards to pathophysiology were challenging to identify. The majority of these papers focused on characterising leukaemic cell identity by exploring what made the leukaemic cells different from their non-leukaemic counter-parts. The majority of such papers inferred that these differences are potential therapeutic targets.

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[12] Among the most impactful risk stratification systems is the European LeukemiaNet (ELN) recommendations, developed by an international expert panel, first published in 2010 and later revised in 2017. In North America the National Comprehensive Cancer Network (NCCN) guidelines is most influential.

***FLT3*-LM AML and the challenges of precision**

FLT3-LM AML is repeatedly portrayed as a classic case of somatic mutation theory and tumour evolution theory, where acquisition of *FLT3*-LMs represents the transition between non-leukaemia and leukaemia (Gilliland and Griffin 2002, Welch, Ley et al. 2012). *FLT3*-LMs are recurrent and as an important haematopoietic regulator altered FLT3 function is a plausible cause of altered cell behaviour. *FLT3*-LMs correlate with cell and disease phenotypes as well as with outcome. In cell and animal model systems expression of *FLT3*-LMs result in AML-like cell behaviour, which is suppressed by FLT3 inhibition, and in combination with specific gene variants *FLT3*-LMs are both necessary and sufficient to generate acute leukaemia in mice. Despite these lines of evidence acquisition of *FLT3*-LMs poorly delineates leukaemic transitions in AML patients. Despite the low abundancy of somatic variants in AML patterns of convergent molecular evolution in *FLT3*-LM AML is common and the pattern of *FLT3*-LM allele burden frequently suggest that *FLT3*-LM cells are sub-clonal. Within the frames of somatic mutation theory, it can be inferred that mutations not shared by all leukaemic cells at time of diagnosis are not necessary for AML eruption. Plurality of *FLT3*-LM at time of diagnosis conversely suggests that *FLT3*-LMs is in-sufficient for leukaemic transformation.

FLT3-LM AML is frequently treated as a distinct biological entity in preclinical and clinical research as well as in clinical practice (Lagunas-Rangel and Chavez-Valencia 2017). Acquisition and loss of *FLT3*-LMs over the course of single leukaemic trajectories, however, makes them unsuitable both for disease delineation as well as sub-group classification. Accordingly, *FLT3*-LMs are not confined to a single disease category within the current WHO classification system, and are rather dispersed across most sub-categories, though with varying frequency.

Positive *FLT3*-LM status correlates with poor outcome and is integrated in modern risk stratification guidelines leading to risk-adapted treatment decisions (Dohner, Estey et al. 2017, O'Donnell, Tallman et al. 2017). Context-dependency with regards to the predictive strength of *FLT3*-LMs, however, has made the optimal positioning of *FLT3*-LM status as a clinical decision-making tool challenging. Initially *FLT3*-LM patients with recurrent cytogenetic abnormalities were categorised according to karyotype, while cytogenetic normal AML with positive *FLT3*-LM status was categorised as intermediate risk-I in a four-level risk classification system comprising favourable, intermediate-I, intermediate-II and adverse risk, respectively (Dohner, Estey et al. 2010). In the 2017 update the number of risk groups was reduced to three: favourable, intermediate and adverse risk respectively. The risk impact attributed to *FLT3*-LMs in these guidelines is dependent not only on concurrent cytogenetic features but also *NPM1* mutation status. In addition, the relative proportion of *FLT3*-LM alleles in the biospecimen (in relation to *FLT3* wild type alleles) determines the final classification. When the *FLT3*-LM allelic ratio is low and co-occurs

with *NPM1* the prognosis is considered favourable as opposed to adverse-risk characterised by high *FLT3*-LM allelic ratio is combination with negative *NPM1* mutation status.

Despite two decades of preclinical and clinical trials focusing on FLT3 targeted treatment these efforts remained fruitless until very recently. Despite initial responses in some patients the clinical course is characterised by rapid emergence of resistant cell populations characterised by additional point mutations in *FLT3* or mutations in unrelated genes (Man, Fung et al. 2012, Smith, Wang et al. 2012, Baker, Zimmerman et al. 2013, Smith, Paguirigan et al. 2017, McMahon, Ferng et al. 2019, Zhang, Savage et al. 2019). The last few years several trials have, however, demonstrated some clinical benefit related to FLT3 inhibition in various settings in *FLT3* mutated AML. This includes addition of a FLT3 inhibitor as maintenance therapy following standard induction therapy (Stone, Mandrekar et al. 2017) or bone marrow transplant (Burchert, Bug et al. 2018) as well as FLT3 inhibition as a single strategy in relapsed or refractory AML (Cortes, Khaled et al. 2019, Dhillon 2019). Currently ELN suggests that *FLT3* mutated AML patients may be treated with midostaurin in addition to intensive chemotherapy (Dohner, Estey et al. 2017), while the National Comprehensive Cancer Network guidelines for AML (Version 3.2017) suggests that midostaurin is administered as consolidation treatment on day 8-21 after induction therapy with cytarabine and daunorubicin (O'Donnell, Tallman et al. 2017). Despite FLT3 treatment is currently included in treatment guidelines the added benefit is sobering. The RATIFY trial found that the 4-year over-all survival rate was 51.4% in *FLT3* mutated patients treated with addition of midostaurin maintenance therapy as compared to 44.3% in the placebo group (Stone, Mandrekar et al. 2017). In the QuANTUM-R trial, comparing quizartinib with salvage chemotherapy in relapsed or refractory *FLT3*-LM AML, Cortes et al. recently observed a survival benefit in the quizartinib group, where median overall survival was 6.2 months compared with 4.7 months in the salvage chemotherapy group (Cortes, Khaled et al. 2019). In a similar trial gilteritinib treatment was shown to prolong survival in relapsed or refractory *FLT3* mutated AML. The median overall survival was 9.3 months in the gilteritinib treated patient group as opposed to 5.6 months in the group treated with salvage chemotherapy (Perl, Martinelli et al. 2019)

Summing up so far: Theories, precision and friction

The literature review presented above has shown that a variety of explanatory models are represented in the AML literature, spanning all levels of biological organisation, ranging from genes to host extrinsic factors. The majority of explicit and implicit conceptual frameworks in the reviewed literature were characterised by strong directionality: the flow of information *from* genes *to* cell behaviour. The simplest version of these frameworks is somatic mutation theory, which proposes that acquisition of mutations is the cause of cancer. Epigenetic models expand on this

assumption by including changes in heritable gene regulation patterns, and tumour evolution theory further develops the biological model by proposing that several mutations and/or epimutations are required for cellular transformation.

The alignment of cancer stem cell theory with somatic mutation theory and its related models is slightly more complex. Cancer stem cell theory holds that the cell in which the mutations and epimutations occur and accumulate, possesses or acquires self-renewal properties and that such properties only characterise a fraction of the cells. What follows is that gene variants translate into phenotypic variation as a function of differentiation, complicating the linear flow of information from genotype to phenotype and, thus, challenging the categorical value of (epi)gene-variants. Explanatory models focusing on how the environment influences phenotype and genotype emergence further suggest that gene variants translate into variation as a function of individual dynamic connectivity. This perspective comes into conflict with the dominating model, reversing the direction of the causal chain.

In the reviewed papers where both a causal narrative and one or several precision medicine related approaches were articulated, the clinical application frequently related directly to the causal narrative. Specifically, the majority of precision oncology related approaches identified were anchored to a gene-centred monocausal narrative. However, the case of *FLT3*-LM AML poses challenges related to somatic mutation theory and tumour evolution theory with regards to all identified precision oncology related approaches, including disease delineation, disease stratification, precise predictions as well as targeted therapy. In other words, there is a friction and a tension present in the case of *FLT3*-LM AML, between on one hand, what is known empirically about the phenomenon, and on the other, explicit and implicit theorisation as well as the in the choice of clinical approaches that are being devised. This discrepancy can be seen as the main subject of investigation in this dissertation.

Aims

In the preceding part of the introduction, I explored the relationship between precision oncology related approaches and conceptual frameworks in AML with focus on *FLT3*-LMs. This exploration concluded with a discrepancy between, on one hand, certain empirical features of *FLT3*-LM AML, and the theoretical and clinical approaches to the condition. The empirical work presented in this thesis represents a further exploration of the discrepancy.

1. In **paper I** we expand on two independent observations from the *FLT3*-LM AML field that are poorly conceptually accounted for within the premises of somatic mutation theory: the sub-clonal as well as plural distribution of *FLT3*-LMs at time of diagnosis. We explore the relationship between *FLT3* variant allele distribution and clinical characteristics, molecular features and outcome.

2. In **paper II** we investigate and describe the relationship between sex and *FLT3*-LM status.

3. In **paper III** we perform an in-depth interrogation of the disease course of a single *FLT3*-LM AML index patient, exploring the relationship between genotype, phenotype and the environment.

Methodological considerations

This work is the synthesis of descriptive, experimental, and analytical practices. Full methodological accounts of the empirical work are presented in the method sections of the respective papers. This chapter focuses on more general methodological considerations and limitations.

Study design

Paper I is a retrospective biomarker cohort study where relationships that frequently are attributed value in the delineation of AML sub-groups are assessed. A series of correlative analysis of biological and clinical variables, in addition to time to event analysis are performed. Therapeutic interventions influence disease trajectories and outcome in AML. Identified prognostic features within a study population consequently reflect population specific treatment practices. Over time identification of prognostic features has resulted in disease sub-stratification and non-random divergence of disease management. This means that when a biomarker is implemented in a treatment algorithm its "original biological signal" becomes in-assessable. It is, thus, unclear to what extent comparison between various AML strata in contemporary AML cohorts reflect variation in biology or variation in clinical care. *FLT3*-LMs have been accepted as negative prognostic markers for almost two decades, and *FLT3*-LM status is routinely implemented in clinical decision making. A recent publication demonstrated reduction of the prognostic impact of *FLT3*-LMs over time, implying relevant impact of risk-adapted practices (Badar, Kantarjian et al. 2015). We are primarily interested in biological signals, an objective best explored in selected, uniformly treated and well characterised patient groups. We further focus on exploring differences within the *FLT3*-LM group, assuming they have been treated equally. We assess two independent and temporally separated cohorts, one representing a homogenous bio-bank cohort where *FLT3*-LM status was retrospectively assigned, and one cohort more representative for contemporary practice, delineated by inclusion criteria of a clinical trial.

In paper II we mostly explore publicly available datasets. Technological advances and novel organisational structures in research, including formation of large collaborative consortiums like the cancer genome atlas (TCGA) and more recently Beat AML, has allowed large cohorts of AML specimens to be analysed across multiple platforms (Cancer Genome Atlas Research Network 2013, Tyner, Tognon et al. 2018) with raw and pre-processed data being provided for exploration by the research community at large. We mainly leverage these two datasets for a comparative analysis of female and male *FLT3*-LM AML patients. While the LAML-TCGA cohort is a highly selected sample population the Beat AML sample cohort represent a consecutive and unselected AML population. Importantly both these cohorts are vastly heterogeneously treated, suggesting

limited value with regards to time of event analysis. Exploration of the prognostic value of *FLT3*-LM status was for this reason expanded to include the two cohorts analysed in paper I.

Paper III is less conventional in structure and content as it presents an in-depth study of the disease trajectory of one single patient, performed with a suite of traditional as well as emerging precision medicine related practices. This includes molecular profiling as well as functional characterisation through drug sensitivity and resistance testing as well as repopulation assays. Knowledge generated by exploring a single case may have limited general value in the terms of its prospects for generalization. It does, however, offer the possibility of exploring and presenting biological complexity which might be invisible when averaging between individuals.

Sample material and sample management

The three studies included in this dissertation involve characterisation of leukaemic biomaterial acquired from peripheral blood draws or bone marrow aspirates of AML patients. In paper I and II samples were collected at inclusion in study protocols or biobanks. In paper III serial sampling was performed and bio-banked in the context of clinical care. In contrast to the vast spatial heterogeneity in solid cancers there has been a general assumption that leukaemic cells are uniformly distributed amongst various bone marrow compartments and between the bone marrow and peripheral blood [13] . The leukaemic biomass, however, comprises genotypically and functionally diverse cell-populations questioning the validity of this assumption. Functional differences in composition of leukaemic cells derived from bone marrow and peripheral blood was noted already in the 1960s, and has lately been expanded on (Mauer and Fisher 1963, Weinkauff, Estey et al. 1999, Cheung, Chow et al. 2009, Sellar, Fraser et al. 2016, Wang, Sanchez-Martin et al. 2017). Recent observations suggest tissue relevant variability in genotype composition (Klco, Spencer et al. 2014, Ojamies, Kontro et al. 2018). Moreover, results from imaging studies using modalities like positron emission tomography have demonstrated uneven anatomical distribution of AML (Buck, Bommer et al. 2008, Vanderhoek, Juckett et al. 2011). The tissue origin in our studies is inconsistent, perhaps reflecting the inherent trade-offs related to clinical research. Bone marrow aspirates are associated with pain and risk of complications, while in some patients the number of circulating blasts is so low that peripheral blood draws are unsuitable due to low leukaemic content. For our purposes, however, Tong et al. have demonstrated complete concordance for detection of *FLT3-LMs* across paired peripheral blood and bone marrow samples,

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[13] The Beat AML exome sequencing data set (Tyner, Tognon et al. 2018), studied in paper II, comprise a sample subset of peripheral blood samples and bone marrow aspirates drawn on the same day (n=14). As a reference I provide supplementary figure 2 demonstrating the level of consistency regarding variant calls by pairwise comparison of these samples.

as well as highly correlated allelic ratios (Tong, Sandhu et al. 2015). We did not observe any significant difference of numerical or allelic distribution in relation to sample origin (data not shown). Sample heterogeneity has further been shown to influence biomarker assessment (Pogosova-Agadjanyan, Moseley et al. 2018), and heterogeneous sample composition may result in non-random phenotypic and genotypic shifts by sample management procedures, including isolation, storage, thawing and expansion (Cantu, Dong et al. 2018).

Genomic characterisation

The characterisation of *FLT3*-LM status in AML is routinely performed by polymerase chain reaction amplification, fragment analysis by capillary electrophoresis and Sanger sequencing. The polymerase chain reaction conditions influence the sensitivity of this analysis. The ratio between *FLT3*-LM and non-mutated patients in AML, thus, reflect assay sensitivity rather than the "true" naturally occurring distribution. While most articles discussing *FLT3*-LM AML state that the mutation occurs in approximately one third of AML patients, assessments by more sensitive methods have revealed that the proportion of *FLT3*-LM patients is substantially larger (Shouval, Shlush et al. 2014). When present at recurrence it has been demonstrated that it often is retrospectively detectable in the diagnostic sample when assessed with more sensitive assays (Ottone, Zaza et al. 2013, Zuffa, Franchini et al. 2015). Determination of the number of *FLT3*-LMs is also strongly influenced by technology, where more sensitive assays detect higher numbers of *FLT3*-LMs per patient (Schranz, Hubmann et al. 2018, Blatte, Schmalbrock et al. 2019). A recent report presented a novel bioinformatic pipeline annotating *FLT3*-LMs from deep next generation sequencing experiments. They reanalysed 28 *FLT3*-LM AML samples, previously characterised by conventional fragment analysis, and identified a total of 105 unique *FLT3*-LMs with a median of 3.8 LMs per sample. In comparison fragment analysis had only detected 34 LMs in the same sample set. Importantly, they analysed several samples from the same individuals at disease presentation, at remission and at disease recurrence and they demonstrated that LMs present in the diagnostic samples re-emerged at relapse despite mutation clearance at remission, suggesting persistence of mutated cells below the sensitivity threshold (Blatte, Schmalbrock et al. 2019).

In paper I we explore the relationship between *FLT3*-LM distribution and molecular *FLT3*-LM features related to clinico-pathological characteristics as well as outcome. Based on the relationship between *FLT3*-LM and non-*FLT3*-LM alleles, estimated by fragment analysis by capillary electrophoresis, we made several assumptions related to *FLT3*-LM cell distribution. Based on sample availability the analysis was performed on complementary DNA rather than genomic DNA. We are hence measuring the relationship between various expressed *FLT3* alleles rather than cellular composition. In a subset of samples, we did, however, demonstrate a very strong

correlation between variant relationships in complementary and genomic DNA, suggesting that complementary DNA variant distribution largely reflects cell population composition. To our knowledge more than one discrete *FLT3*-LM has never been reported to co-occur within the same cell in AML. On the contrary, results from single cell sequencing studies indicate that different LMs represent discrete cellular subsets (Paguirigan, Smith et al. 2015). We accordingly assumed that plurality of *FLT3*-LMs represent plurality of cell populations that independently acquired *FLT3*-LMs. We further assumed that single *FLT3*-LM were restricted to one allele as long as the total variant distribution was below 50%. This may not be the case as loss of heterozygosity is well described in this locus in AML (Thiede, Steudel et al. 2002, Stirewalt, Pogosova-Agadjanyan et al. 2014, Loke, Akiki et al. 2015), resulting in potential overestimation of the *FLT3*-LM cell fraction.

In paper II and III we analyse and present results from next generation sequencing experiments. Whole-exome sequencing is a capture-based next generation sequencing method that is designed to cover the known protein coding part of genomic DNA. The aim is to produce high-quality, unbiased and interpretable data. The optimal conditions are achieved at uniformity of coverage and complete breadth (percentage of target bases that are sequenced a given number of times of the target bases). There are, however, several technical and biological challenges related to the method that tend to result in significant error rates. The source of errors can be related to sample representativeness, sample management and pre-processing, polymerase chain reaction errors through amplification, reference bias (capture probes that match the reference sequence and thus tend to preferentially enrich the reference allele at heterozygous sites), unmapped reads (low quality, sequencing errors, structural rearrangements, insertions in the query genome, deletions in the reference) and mis-mapped reads due to low complexity sequences. Increasing read length decreases the risk of mis-alignment. One major challenge is that GC-rich regions (CpG islands) are difficult to amplify as they remain annealed during amplification. These regions are hence prone to low coverage, which may result in gaps in the sequences and possibly false negatives. GC content variation also influences the hybridisation efficiency of sequence capture probes which may result in target regions that have little or no coverage. The confidence of variant whole-exome sequencing calls is in part based on discrimination between the sample of interest and a control. Application of whole-exome sequencing in search of postzygotic variants is consequently complicated by contamination of the control sample by leukaemic cells or cells from blood donors or bone marrow donors as well as the heterogeneity of the leukaemic sample. Coverage of the control sample is also an important feature as it influence statistical outcome (Sims, Sudbery et al. 2014).

Next generation sequencing is perhaps the technology that thus far has provided the strongest data on clonal composition. Bulk analysis of AML genomes is, however, constrained by sample

diversity. The more composite the sample, the fewer mutations are called because of low frequency variants resulting in a strong bias towards detecting the most prevalent variants in a sample. High frequency of false positive and false negative calls makes it unreliable when attempting to assess rare cellular subsets (Sims, Sudbery et al. 2014).

In paper III we perform both whole-exome sequencing as well as targeted sequencing on samples derived from patient derived xenograft models. Both methods are capture based and homologous sequences between murine and human DNA may lead to amplification of and alignment of residual murine DNA, resulting in false positive and false negative reads. For analysis of these whole-exome sequencing experiments we therefor only considered calls that were detected at high confidence in the primary material. For the targeted sequencing we manually investigated the reads for mouse contamination. We found minor amplification of murine DNA in two of the targets resulting in both overestimation and underestimation of variant reads. The relative contribution of murine reads was, however, low and only marginally affecting variant allele frequencies.

Phenotypic characterisation

Differential gene expression analysis

In paper II we questioned whether there were any differentially expressed genes between female and male *FLT3*-LM positive samples in the Beat AML sample cohort. We did so by analysing the RNA sequencing data provided as part of the publication of the cohort (Tyner, Tognon et al. 2018). By conversion of RNA to complementary DNA followed by sequencing and read count quantification of transcripts one can address a variety of questions related to the transcriptome, within and between samples. The experimental and analytic pipeline of RNA sequencing resembles that of whole-exome sequencing and includes both *in vitro* as well as *in silico* procedures (Stark, Grzelak et al. 2019). Reading depth may vary between experiments. For comparison between samples read counts are normalised, like in Beat AML, to counts per million reads (CPM). This metric does not account for variation of counts per gene due to variation in gene length. One can, therefore, not compare expression between genes within the same sample. The comparative analysis performed included more than 20 000 statistical tests representing all identified protein coding genes. With an alpha set at 0.05 one would expect identification of more than 1000 hits by chance alone. We therefor adjusted for multiple testing effects and used a cut of false discovery rate at 5%.

Mass and flow cytometry

In paper III we assess sample composition and relative distribution of various cell populations by means of two technological platforms: flow cytometry and mass cytometry respectively. Both flow and mass cytometry are antibody-based methods permitting synchronous characterisation of multiple molecular features at the resolution of single cells. Technically they differ with regards to labelling reagent as well as data acquisition technology. In flow cytometry experiments cells are stained with antibodies tagged with fluorophores, and fractions of cell populations corresponding to the relative strength of the fluorophore signal is quantified by means of the flow cytometer. Analogously, mass cytometry analysis is based on staining cells with antibodies tagged with rare-earth metal isotopes of varying atomic mass. Relative quantification is subsequently acquired by an inductively-coupled plasma mass spectrometer (Fan, Wang et al. 2018). Our research objectives included i) delineation of non-leukaemic immune cells and leukaemic cells, ii) characterisation of immunophenotypic heterogeneity within the leukaemic cell compartment, iii) assessment of temporal variation in phenotype composition, iv) quantification of human cells in the patient derived xenograft models, and v) evaluation of environmental diversification by comparing leukaemic cells expanded in various organ sites and in discrete mice models. The different objectives required different resolution, which influenced the choice of platform. For phenotypic characterisation we used mass cytometry as it offers a multiplexing approach which is more robust than multi-colour flow cytometry. The analysis is based on a rationally selected panel of anti-bodies comprising canonical and non-canonical cell-surface markers resolving well known normal cell populations as well as the malignant cell compartment. For assessment of relative frequency of human cells in the patient-derived xenograft samples flow cytometry offered sufficient resolution. Both flow cytometry and mass cytometry are associated with technical noise. In mass cytometry contamination of ions from experimental conditions can generate channel specific noise. Analogously, several endogenous cell components produce autofluorescence and background signal at certain wavelengths. In flow cytometry the possibility of multiplexing is limited by wave length distributions of the light emitters. As the number of fluorophores is increased, resolution is limited by spectral overlap, spill-over and false positive signals in neighbouring channels. Mass cytometer sensitivity is isotope specific and signal intensity reflects panel composition. One can therefore only compare signal intensity within the same channel, further, complicated by signal spill-over. Ion instability can result in oxide formation resulting in the addition of 16 mass units. Furthermore, data processing of both flow and mass cytometry experiments tends to result in loss of information. Single cell high dimensional data are gated and analysed by various computational tools including clustering and dimensionality reduction algorithms. Despite a high level of automation several analytical steps comprise elements of subjectivity, including gating, transformation and determination of relationships, together compromising the reproducibility of

final results (Olsen, Leipold et al. 2019). Of particular concern with regards to our analysis we analysed several samples comprising both human and murine cells. For mass cytometry analysis we analysed a stringent anti-human CD45 gate. Loss of CD45 low human cell subsets cannot be excluded. Antibody cross reactivity and lack of specificity may introduce additional noise.

Functional characterisation

Drug sensitivity and resistance testing

In paper II and III we present data from high throughput *ex vivo* functional drug screens. In paper II we analysed data from the Beat AML sample cohort (Tyner, Tognon et al. 2018) where they assayed live AML cells with a colorimetric MTS assay. In paper III we run a luminescent ATP assay (Pemovska, Kontro et al. 2013) on thawed vitally-preserved cells from the index patient as well as on cells derived from the patient derived xenograft models. Such viability assays are designed to test pharmacological dependencies of primary leukaemic cells under preselected conditions. Both selective drug sensitivity scores and area under the curve are measures of relative efficacy and express a function of the interaction of the primary leukaemic cells, the experimental conditions, and the assay specific read out. The experiments provide large amounts of data that can be used to characterise and compare various samples. Results may indicate sample specific variation in activity and dependency of molecular pathways. The preselected conditions, however, including growth conditions, incubation time, and choice of readout, may limit the utility of comparisons between drugs. The read-out of both the MTS and the ATP assay represent a proxy of cell proliferation and viability by estimation of metabolically active cells by quantification of NADH activity and ATP respectively (Gordon, Brown et al. 2018). The assays comprise compounds, however, that in a clinical context may be used in different ways, and they may provide clinical benefit through other mechanisms than inhibition of cellular proliferation. The assay further provides an average response across the cells in the sample and does not account for functional or genotypic heterogeneity within the specimens. An important example of the importance of this limitation was presented by Pratz and colleagues demonstrating that *FLT3*-ITD ratio correlated with *in vitro* responses to FLT3 targeted tyrosine kinase inhibitors (Pratz, Sato et al. 2010). A second objective related to this kind of high throughput platforms is to assess whether functional characterisation of primary patient material can guide therapy decisions and ultimately lead to improved outcomes, as indicated by Marinez-Cuadron and colleagues (Martinez-Cuadron, Gil et al. 2018).

Repopulation assays

In paper III we explore gene gene-environment interactions and how various conditions influence leukaemic initiating potential in repopulation assays. The choice of mouse strain and model system has been shown to influence the pattern of engraftment of human material (Antonelli, Noort et al. 2016, Wunderlich, Chou et al. 2018). We chose two variants of the NOD/SCID strain characterised by discrete features: i) a NOD/SCID line characterised by IL2RG knockout (NSG) and ii) a more humanised NOD/SCID line with IL2RG knockout in addition to transgenic expression of several human myeloid promoting cytokines, including SCF, GM-CSF and IL-3 (NSGS). We included both female and male animals. Leukaemic engraftment rate has been associated with observation time (Paczulla, Dirnhofer et al. 2017). We were predominantly interested in investigating disease initiation and propagation rather than engraftment and, thus, chose to sacrifice the mice at disease onset rather than a predefined timepoint. It has also been demonstrated that different clones distribute in an asymmetric fashion, varying between different skeleton location as well as between the skeleton and extra medullary sites (Belderbos, Koster et al. 2017). Due to scarcity of sample material such heterogeneity is unfortunately poorly accounted for in our analysis.

Statistical considerations

Statistics were performed in line with usual practice in the field. For pairwise comparison of normally distributed continuous variables we performed the Student´s t-test. The non-parametric Wilcoxon signed-rank test was applied for the pairwise comparison of continuous variables which failed to fulfill the requirements of a parametric test. For comparison of categorical variables, we performed 2x2 tables and applied the 2-sided Fisher exact test. Pearson correlation was used to test relationships between continuous variables. For time to event analysis overall survival was calculated by the Kaplan-Meier method and was visualised in Kaplan-Meier plots. The 2-sided log-rank test was applied to compare Kaplan–Meier estimates. For continuous variables impact on outcome was evaluated by univariate cox models. For multivariate adjustment we performed multivariate Cox proportional hazards regression analysis. Statistical significance was considered to be indicated when the p-value was equal to or below a threshold of 0.05. Due to the multiple testing situation p-values were adjusted by the Benjamini-Hochberg method (Benjamini and Hochberg 1995) for differential gene expression analysis. Tests were performed in accordance with test specific requirements, and all the statistical calculations and the graphical representations were performed in R- Studio (version 1.1.453) and R (version 3.5.0). Graphs were made with ggplot2 (version 3.1.0) and figures made in Adobe illustrator CS6 (version 16.0.0).

Results and discussion

Through an appraisal of the conceptual frameworks presented in the AML research literature I have argued that theoretical premises are reflected in precision oncology related practices in AML, and that they predominantly rely on the premises of somatic mutation theory. Evidence supporting i) pervasiveness of somatic diversification, ii) patterns of converging molecular evolution, and iii) context dependency with regards to the qualities of specific gene variants in sum indicate limited explanatory potential with regards to this model. Expanding the *FLT3*-LM AML evidence base with regards to molecular convergence and context dependency I substantiate the relevance of these dimensions. Full accounts of the results are presented in the respective manuscripts. For a brief overview I refer to the abstracts. In concert they support a theory of leukaemia as a relational multidimensional process. In the following I shall discuss the evidence for this claim as well as its potential epistemic and translational implications.

FLT3-LMs – cause or effect?

While leukaemia is frequently described and categorised as an entity our results accentuate that *FLT3*-LM AML perhaps is best understood and described as an evolving process. In paper I we explored the distribution of *FLT3*-LMs in treatment naïve AML. We found that the fraction of the leukaemic disease that is characterised by *FLT3*-LMs is highly variable, ranging from a diminishing portion of the leukaemic cells to defining most of the tumour cell population. In paper III we show that the *FLT3*-LM allele fraction can be unstable over time. We further show that an individual leukaemic disease compartment can entail several *FLT3*-LM negative cell populations that can propagate leukaemia. We find that gene-environment correlate with the molecular manifestation of *FLT3*-LM AML as well as *FLT3*-LM cell properties. Of novelty we observe that relevant gene-context may include sex.

FLT3-LM status is repeatedly found to predict outcome in intensively treated AML (Kiyoi, Naoe et al. 1999, Abu-Duhier, Goodeve et al. 2000, Kottaridis, Gale et al. 2001, Meshinchi, Woods et al. 2001, Frohling, Schlenk et al. 2002, Schlenk, Dohner et al. 2008, Patel, Gonen et al. 2012). Plurality or sub-clonality of *FLT3*-LMs at time of diagnosis as well as context dependency with regards to the translational consequences of *FLT3*-LMs suggests that the relationship between *FLT3*-LM status and disease outcome may be less linear than what is frequently proposed. Although most of the biomedical literature focuses on the direct translational effects of the *FLT3*-LM protein alternative hypothesis are represented in the literature. While some authors have shown that *FLT3*-LMs are inducers of reactive oxygen species and genetic instability (Sallmyr, Fan et al. 2008), others have suggested that *FLT3*-LMs are the result of such instability. *FLT3*-LMs have from the very beginning been suggested to be an expression of DNA replication error (Kiyoi,

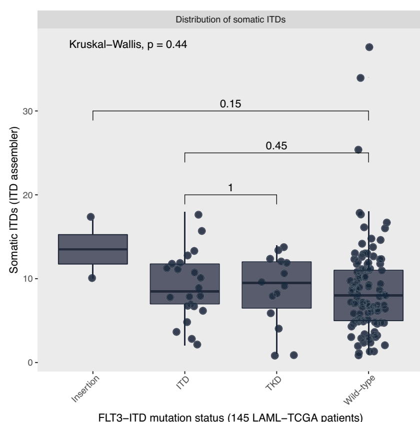

Figure 15: Rustagi et al. evaluated the ITD Assembler on exome sequencing data from 157 AML patients from LAML-TCGA cohort, and the results revealed that somatic ITDs are frequent in AML. The boxplots and analysis is based on data presented in the Supplementary figure 1 provided by Rustagi, Hampton et al. 2016. The patient ID was used to retrieve FLT3-ITD mutation status from the supplementary table "SuppTable01.xlsx" downloaded on https://gdc.cancer.gov/node/876 under the header Supplemental Data from NEJM paper [Supplementary]. The TCGA Patient ID 2825 appeared twice in the figure and was therefore not included in the analysis. The number 2852 appeared in the figure but does not correspond to a TCGA Patient ID and was therefore removed from the analysis. A total of 145 cases remained and are included in the analysis. Pairwise analysis are performed by the unpaired two-samples Wilcoxon Test.

Towatari et al. 1998), and identification of plural *FLT3*-LMs early on led to the hypothesis "…that *FLT3* aberrations are potentially not the causative event but the result of an inherent genetic instability of the leukaemic cells due to an as yet undefined defect" (Thiede, Steudel et al. 2002). It has later been suggested that *FLT3*-LMs may result from genetic instability induced by preceding mutations in genes coding for epigenetic modifiers (Wakita, Yamaguchi et al. 2013). Accumulating data comparing the load and composition of mutations in *FLT3*-LM AML and non-*FLT3*-LM AML, however, suggest that the number and pattern of somatic variants is similar (Garg, Nagata et al. 2015, Tyner, Tognon et al. 2018) reducing the probability of genetic instability as an important causal contributor.

The different prognostic impact of *FLT3*-LMs and *FLT3*-TKD mutations, both resulting in constitutive activated kinase activity, has been a persistent conundrum in the AML literature (Mead, Linch et al. 2007). One explanation could be that the molecular mechanisms involved in LM formation confer prognostic impact, recently indicated by some authors (Borrow, Dyer et al. 2019). While somatic ITDs rarely are reported in the literature the mechanism has been described in other genes in relation to malignancy. *cKIT*-ITDs are frequent findings in canine cutaneous mast cell tumours (Webster, Yuzbasiyan-Gurkan et al. 2006), and ITDs of the *EGFR* kinase domain characterise the majority of soft tissue tumours in infants (Wegert, Vokuhl et al. 2018). *BCOR*-ITDs have been described in clear cell sarcoma of the kidney (Roy, Kumar et al. 2015), soft tissue undifferentiated round cell sarcoma of infancy (Kao, Sung et al. 2016), and in high-grade uterine sarcomas (Marino-Enriquez, Lauria et al. 2018). ITDs are bio-informatically challenging to identify in next generation sequencing data, suggesting that they are underreported. Novel analytic algorithms have facilitated ITD detection in such datasets. Rustagi et al. quantified the number of somatic ITDs in the LAML-TCGA cohort (Rustagi, Hampton et al. 2016), showing that ITDs are frequent somatic events across most AML cases. Analysing these data with respect to *FLT3*-LM

status no correlation between the number of somatic ITDs and *FLT3*-LM status were found (Figure 15), indicating that such a mechanism is unlikely.

In the following section, based on observations from the empirical work presented in the three research papers included in this thesis and from a selection-centred perspective an alternative explanatory model that might account for the persisting relationship between *FLT3*-LM status and outcome will be explore.

Somatic diversification and a selection-centred perspective

Convergent molecular evolution is a recurring observation in the empirical work of this thesis. The plurality of *FLT3*-LMs in AML as presented in paper I and the dual *NRAS* mutations characterising the index patient presented in paper III both illustrate intra-individual molecular convergence. Inter-individual convergence is exemplified by the sex-associated mutation patterns presented in paper II, and the recurrent engraftment of a rare molecular defined cell-population described in paper III.

According to somatic mutation theory and tumour evolution theory AML is the result of acquisition of somatic variants followed by clonal selection. While the field has emphasised the impact of mutations as the source of selective benefit evolutionary theory suggests that convergence predominantly is the result of environmental constraints (Losos 2011). The arguments and results of this thesis question whether changes in selection pressure plays a more important role in leukaemogenesis than what is currently acknowledged in the research literature, as assessed in the literature review presented in the introduction. This claim is substantiated by recent observations from the study of treatment related AML. The onset of AML in individuals that have undergone genotoxic treatment has been considered strong supportive evidence of the importance of mutagenesis in AML pathogenesis. Recent reports, however, suggest that chemotherapy primarily promote leukaemogenesis through selection rather than by induction of mutations. Risk of developing treatment related AML is correlated to the presence of clonal haematopoiesis prior to initial chemotherapy (Gillis, Ball et al. 2017, Takahashi, Wang et al. 2017), and *TP53* mutations have been reported to frequently be present 3-6 years prior to *TP53* mutated AML presentation. In some cases, *TP53* mutations have even been identified before administration of genotoxic therapy. Treatment related AML is further characterised by a similar mutation pattern as the one found in chemotherapy naïve patients, and the number of mutations does not exceed the number identified in *de novo* AML (Wong, Ramsingh et al. 2015). In light of this, one could speculate whether *TP53* mutations primarily provide a selective benefit in a setting where a *TP53* response is induced, for example by chemotherapy (Anensen, Oyan et al. 2006, Oyan, Anensen et al. 2009). Similar observations, although anecdotal, have been reported in both *NRAS* and *FLT3*-LM AML. This

includes a report describing the emergence of *RAS*-mutated leukaemia in a patient treated with a BRAF inhibitor for a malignant melanoma (Callahan, Rampal et al. 2012), and a paper presenting the manifestation of a *FLT3*-LM AML in relation to dis-continuation of sorafenib (tyrosine kinase inhibitor with FLT3 affinity) treatment for an unrelated papillary thyroid cancer (Kakiuchi, Yakushijin et al. 2018). The natural history of AML is reflected in mutational profiles (Lindsley, Mar et al. 2015). Similar observations have been made in solid cancers, linking gene mutation status with exposure. Distribution of driver mutations in lung cancer reflect historical smoking status (Mounawar, Mukeria et al. 2007), while the recurrent somatic variant pattern in liver cancer displaying biliary phenotype is associated with aetiology, differing between individuals with and without antecedent chronic hepatitis (Fujimoto, Furuta et al. 2015). Analogously, the mutation spectrum between sporadic and inflammatory bowel disease associated colorectal cancers has been shown to diverge (Robles, Traverso et al. 2016). In concert these observations suggest that genotype-emergence can be a function of gene-gene-environment interactions (Temko, Tomlinson et al. 2018). Thus, patterns of molecular convergence between individuals and within individuals could suggest prior or current shared ecological conditions.

The cancer cell – a confined entity or a potential property?

In 1975 the embryologist Beatrice Mintz demonstrated reversal to non-malignancy of malignant mouse teratoma cells. Through integration into normal mouse blastocystes and subsequent orderly embryonic development the malignant cells integrated in the growing animal, resulting in a mosaic of healthy and normal functional tissue (Mintz and Illmensee 1975). This experiment is an elegant demonstration of how cell population trajectories are in part determined by environmental conditions. Traditionally cell identity has been understood as confined end-states of ordered unidirectional sequences of branching steps (Ferrell 2012). Cancer cells have consequently been considered designated actors. The emergence of genotypes as a result of cell extrinsic events or processes, however, challenge the categorical boundaries of cancer cells.

Emergence of descriptive and experimental data has suggested a previously unrecognised degree of fluidity and plasticity among cell types (Nestorowa, Hamey et al. 2016, Karamitros, Stoilova et al. 2018). By transduction of four distinct transcription factors Takahashi and Yamanaka challenged the unidirectionality of cell fate trajectories, demonstrating that somatic human fibroblasts could be de-differentiated to pluripotency (Takahashi, Tanabe et al. 2007). Riddell and colleagues have similarly shown that this is possible within the haematopoietic system. Expression of six defined transcription factors was sufficient to induce haematopoietic stem cell properties in committed murine blood cells (Riddell, Gazit et al. 2014). Reversal of cell identify and gain of stemness properties has also been shown as a response to stress (Milanovic, Fan et al. 2018), and

under certain conditions leukaemic cells characterised by loss of tumorigenic properties have been shown to require self-renewal properties (McKenzie, Ghisi et al. 2019). Based on this experimental evidence Laplane has presented a conceptual analysis suggesting that stemness as a cellular property may not be best represented by stem cells as confined entities, but rather as cells capable of stemness function (Laplane 2015). Founded on observations from our own work, in light of the evidence mentioned above and the conceptual analysis related to stem cells and stemness my claim is that it may not be unreasonable to think about cancer cells in similar terms, not as cellular entities but as cells capable of “cancer” or “neoplastic” function.

Intra-leukaemic heterogeneity and clonal dominance

Results from next generation sequencing of bulk AML biomaterial has demonstrated that mutations in signalling genes usually are mutually exclusive, and that the majority of AML patients are characterised by only one mutation in such a gene (Cancer Genome Atlas Research Network 2013, Tyner, Tognon et al. 2018). Several studies, however, have demonstrated that at time of diagnosis AML frequently comprise multiple genetically discrete cell populations (Klco, Spencer et al. 2014, Shouval, Shlush et al. 2014, Paguirigan, Smith et al. 2015), indicating that bulk AML biomass assessment poorly captures the complexity of cell population composition. The multiplicity of *FLT3*-LMs in a substantial fraction of the *FLT3*-LM AML cases (Paper I), as well as the diversity of discrete genotypically defined cellular populations identified in the index patient (Paper III) underscores the relevance of this underestimation. Recurrent expansion of non-*FLT3*-LM cell populations harbouring mutations in other signalling genes following treatment with FLT3 inhibitors further highlights that the index patient is representative, and that composite disease composition is clinically relevant (McMahon, Ferng et al. 2019, Zhang, Savage et al. 2019). The dominance of single genotypically defined cell populations, nevertheless, suggests uneven contribution of leukaemic propagation. In paper III we describe a patient derived cell population, characterised by a *NRAS* Q61L variant, that despite temporal persistence never became dominant in the patient from which it derived. Repeatedly, though, cells from this population engrafted and propagated leukaemia in the mouse models. This indicates that shifts in ecological conditions, either by changes in the composition of the leukaemic and pre-leukaemic biomass or as a direct consequence of the treatment, may alter the relative fitness of various genotypes.

Latency and emergence of secondary malignancies in relation with non-genotoxic therapy

The pattern of multiple *FLT3*-LMs in AML (Paper I) is strongly suggestive of intervals of latency where cells have acquired *FLT3*-LMs but do not express leukaemic properties. Perhaps the strongest evidence of neoplastic capability of such latent cells is the emergence of secondary

cancers in relation to treatment with non-genotoxic cancer agents, as the *NRAS* mutated and *FLT3*-LM leukaemia described above, which both presented in relation to tyrosine kinase inhibition (Callahan, Rampal et al. 2012, Kakiuchi, Yakushijin et al. 2018) (Figure 16). Caution in interpreting these results are warranted as they represent single disease narratives, but the mechanism is not leukaemia specific and is supported by observation from other haeme-malignancies and management of solid cancers. Expansion of B-cell clones and development of aggressive B-cell lymphoma in patients with myeloproliferative neoplasms treated with JAK1/2 inhibitors have been reported (Porpaczy, Tripolt et al. 2018). It is not clear whether this can be attributed to the release of neoplastic properties as a direct effect of JAK1/2 inhibition, but it remains a possible explanation. Treatment with tyrosine kinase inhibitors like vemurafenib, a selective RAF inhibitor, is associated with emergence of *RAS*-mutated tumours unrelated to the primary cancers, including but not limited to squamous cell skin cancer (Boussemart, Routier et al. 2013, Gibney, Messina et al. 2013). The specific mutational pattern suggests a direct mechanism where *NRAS* mutated cells with a malignant but constrained potential acquired a selective benefit as a consequence of *RAF* inhibition leading to emergence of neoplastic properties.

Spontaneous remissions, late recurrences and donor cell leukaemia

Although highly unusual, spontaneous AML remissions have been reported in the literature, including in *FLT3*-LM AML (Rashidi and Fisher 2015, Vachhani, Mendler et al. 2016). Whether such disease courses illustrate clearance of leukaemic cells or reversal of leukaemic properties is not known. Durable remissions followed by disease recurrence, however, are suggestive of long-term persistence of cells with leukaemic potential. Although disease recurrence in AML usually occurs within a timeframe of 2 years relapse after durable disease-free intervals have been documented. Yilmaz and colleagues demonstrated that after a median latency of 7 years after initial remission 8 of 10 AML re-presentations were characterised by the initial founder mutation (Yilmaz, Wang et al. 2019). Farina and collegues presented a case report demonstrating longitudinal complex clonal dynamics following complete heamatological remission of AML. Through analysis of samples aquired over 22 years they described alternating expansion of various cytogenetically defined cell population without clinical signs of disease (Farina, Rossi et al. 2016). A recent case study described the presentation of a *BCR-ABL1* mutant cell population presenting as a B-cell precursor acute lymphoblastic leukaemia in a young child. The leukaemia was successfully treated but 22 years later the patient developed AML, genetically characterised by the initial translocation (Ford, Mansur et al. 2015), demonstrating the time frame of which cells with leukaemic potential can persist without propagating disease. Donor cell leukaemia may further be accounted for in this model, where cells from a donor progress to re-initiate leukaemia in the recipient. This has been thought of as a very rare mechanism, but recent advancements in

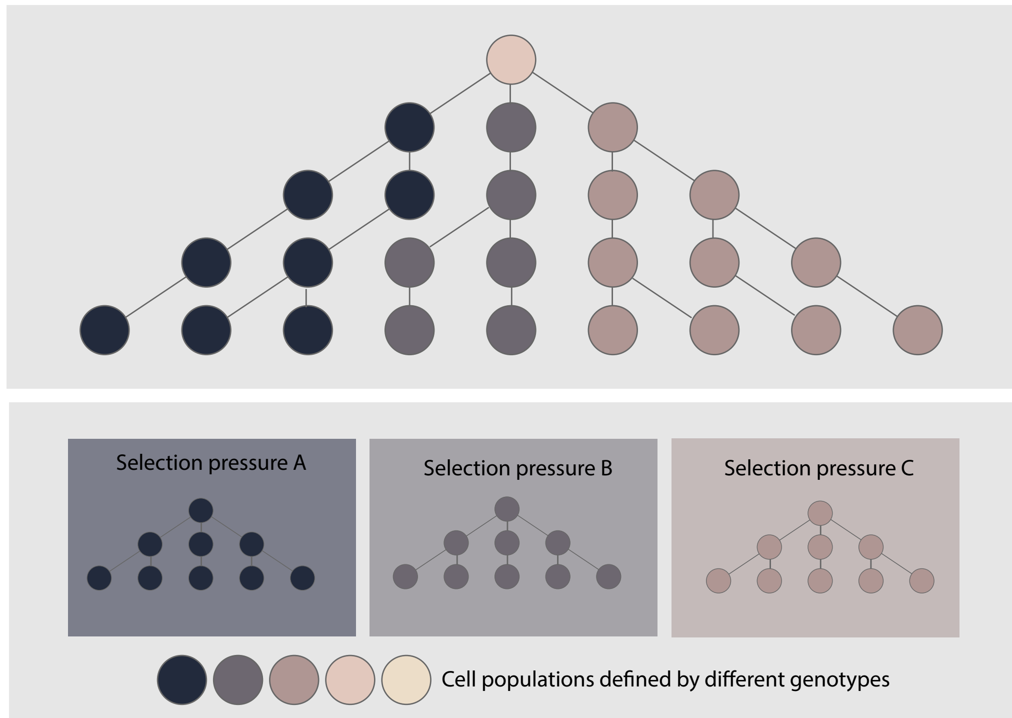

Figure 16: According to somatic evolution theory DNA integrity is continuously broken resulting in gradually increasing somatic genetic diversification. How novel genetic configurations influence fitness is a function of the gene-gene environment interaction. Based on observations of AML disease courses one could hypothesise that changes in environmental conditions lead to redirection of cell population trajectories. Selection pressure A may be a BRAF inhibitor resulting in emergence of NRAS mutated leukaemia. Selection pressure B may be a FLT3 inhibitor resulting in presentation of a FLT3-LM leukaemia, while selection pressure C may be genotoxic chemotherapy resulting in emergence of TP53 mutated leukaemia.

technology has revealed that up to 5% of post-haematopoietic stem cell transplant relapses are attributable to expression of leukaemic properties in cells derived from the graft (Wiseman 2011).

Persistence of cell populations characterised by oncogenes in healthy individuals

Data from the study of chronic myeloid leukaemia and myeloproliferative neoplasms further support the relevance of gene-context dependency with regards to expression of neoplastic properties, both in relation to age and sex. Chronic myeloid leukaemia has frequently been attributed to the acquisition of the *BCR-ABL* fusion proteins, largely reliant on the relationship between expression of *BCR-ABL* and myeloproliferative phenotypes in animal models (Daley, Van Etten et al. 1990). *BCR-ABL* (p210) transcripts are, however, not infrequently expressed in healthy individuals (Ismail, Naffa et al. 2014), and while incidence of chronic myeloid leukaemia was substantially increased in Hiroshima A-bomb survivors, latency before disease onset was in the range of 10 to 40 years. Interestingly, the latency was three times longer in females compared to

males, corroborating the hypothesis of context dependency that includes sex-variation (Radivoyevitch, Jorgensen et al. 2019). Similar patterns are related to myeloproliferative neoplasms. While expression of *JAK2* V617F result in myeloproliferative phenotypes in mice (Li, Spensberger et al. 2010) the same somatic variants have been described in the blood of healthy individuals (Acuna-Hidalgo, Sengul et al. 2017). Disease phenotype and mutational patterns also vary by age and sex (Spivak 2017).

Leukaemogeneic transitions and limitations of somatic mutation theory

Plurality of genetically discrete AML cell populations at time of diagnosis (Paper I/Paper III) can be accounted for within the paradigm of somatic mutation theory. AML can be considered a biphasic disease, characterised by a latency phase followed by a bone marrow failure phase, as proposed by Shlush and Mitchell (Shlush and Mitchell 2015). Based on the findings put forward in this thesis, however, I propose the alternative explanation that the transition from pre-leukaemia to leukaemia involves synchronous emergence of leukaemic properties in multiple cells.

Based on the close relationship between precision medicine and the premises of somatic mutation theory I believe it is important to know if plurality of cell populations characterised by discrete “driver” mutations represent sub-clonal diversity or poly-clonality (Figure 17). Numerous driver mutations as an expression of sub-clonal diversity imply that discrete events occurred independent of each other after the leukaemia was initiated. Their contribution to single cell phenotypes is necessarily not required for the leukaemic property of single cells, but rather result in additional variation, perhaps influencing competitiveness throughout the disease course. In concert i) the rapid clinical onset of AML, ii) the limited number of somatic variants characterising the disease, and iii) observations suggesting relative stability of number of somatic variants across disease courses, suggest that if discrete driver mutations represent sub-clonal cell populations then AML is likely subclinical for a very long time prior to symptomatic presentation. The temporal resolution related to clonal heterogeneity and disease presentation is poorly understood. Welch and colleagues, however, observed that in patients with multiple sub-populations of cells within the leukaemic specimen the average number of mutations specific for what they defined as sub-clones was 40 (range 6-110) as determined by whole-genome sequencing (Welch, Ley et al. 2012). Based on the estimated timeframe for which mutations accumulate this indicate that the sub-clones separated from the founder clone on average 4 years prior to disease presentation (range 6 months – 10 years).

Under the assumption that i) mutated genes only translate to aberrant cell behaviour as a function of their relational connectivity and ii) that this network of relations is in constant motion, a mutated gene will only contribute in the expression of leukaemic properties under certain conditions. It is of importance to determine if it is prospectively possible to determine if a cell population

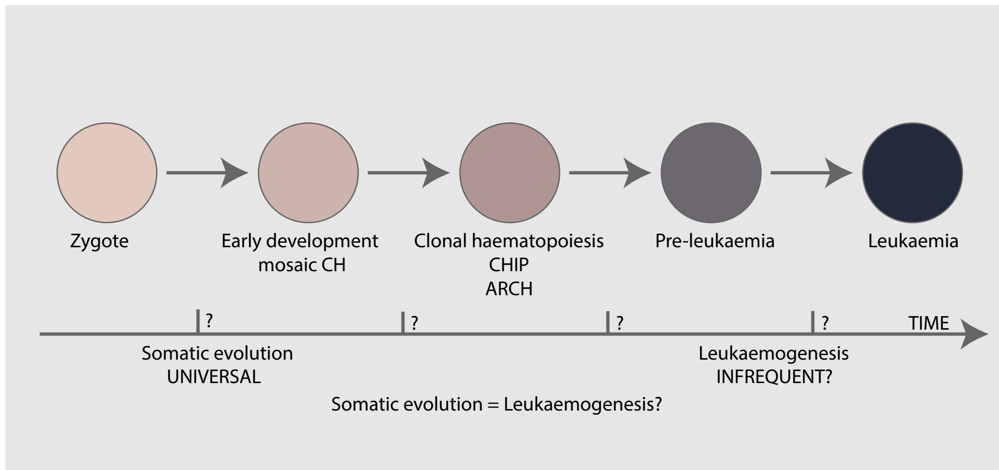

Figure 17: The monoclonal origin of cancers is a central premise in somatic mutation theory. AML is also frequently referred to as a «clonal» disease. It is, however, unclear from the literature selection how this is to be interpreted with regards to tumour evolution theory. It is even less clear considering the emerging evidence in support of somatic evolution. The question is whether the transitions between CH and pre-leukaemia or pre-leukaemia and leukaemia are monoclonal or if the "monoclonal" origin of leukaemia is traced back to the "initiation" of CHIP. Essentially every human body is «clonal», as every single cell asexually descends from a single cell. Terms that are frequently represented in the literature include "founder clone", "leukaemia initiating cell", "leukaemia propagating cell", "transformed cell", or mutations related to "initiation" or "progression".

CH: Clonal haematopoiesis, CHIP: clonal haematopoiesis of uncertain potential. ARCH: age related clonal haematopoiesis.

characterised by a gene-variant is destined for leukaemic propagation in the future. Only if this is possible a leukaemic latency phase can be considered "true" without expanding the category of AML to include individuals that would otherwise never progress to clinically relevant disease, perhaps even the entire population. The problem, however, is that there is no reliable way to forecast future fluctuations of gene-environments, either as forthcoming acquisition of novel somatic variants or systemic alterations such as immune or stress responses or exposure to chemical or pharmacological constraints (Laplane, Duluc et al. 2018).

Conversely, plurality of driver mutations as an expression of poly-clonal disease presentation suggest that the mutations were not sufficient for the establishment of an AML disease phenotype. To my knowledge *FLT3*-LMs have never been described in clonal haematopoiesis or in healthy individuals. *FLT3*-LMs can, however, persist in the bone marrow at levels far below standard assays during remission (Blatte, Schmalbrock et al. 2019), suggesting that the fact that they are not detected does not imply that they are not there. It further signifies that an event at a higher level of organisation than single cells eventually resulted in emergence of leukaemic properties across discrete pre-leukaemic cell-subsets concurrently. This indicates that proximate cause of leukaemia may be distinctly different from acquisition of somatic mutations and that leukaemia may not originate from a single cell. This contrasts the assumptions and premises of somatic mutation theory.

In light of this hypothesised trajectory it is not impossible that the prognostic impact of *FLT3*-LM status may be confounded by shared underlying features facilitating the emergence of *FLT3*-LMs. This could account for why high *FLT3*-LM variant distribution is a stronger predictor of poor outcome that for individuals characterised by single or plural *FLT3*-LMs as described in paper I. Notably, a similar observation has been made in core binding factor leukaemia, where expansion rather than detection of *cKIT* mutated cells predict poor overall survival (Allen, Hills et al. 2013). As with *FLT3*-LMs *cKIT* mutations in this context are frequently lost at relapse (Allen, Hills et al. 2013), and higher sensitive assays have demonstrated that plural *cKIT* mutations at time of diagnosis is a recurrent finding (Jang, Yoon et al. 2016).

We do not know which conditions that promote emergence of *FLT3*-LM mutated cells and simultaneously account for poor outcome. We may, however, speculate with basis in the biological understanding. FLT3 expressing haematopoietic progenitor cells are in part dependent on FLT3 activation for expansion. One could hypothesise that a bone marrow environment chronically or transiently failing to sustain FLT3-ligand production, locally or systemically, could produce a selective benefit for *FLT3*-LM cells. An alternative explanation may be that *FLT3*-LM cells continuously explore leukaemia-like phenotypes. Emergence of such cells may, however, be supressed by a negative selective pressure imposed by the immune system which continuously suppress transcriptionally deviating cells. A progressive or transient deregulation of such an immune response could consequently results in abrupt expansion of several *FLT3*-LM cells synchronously. Such shared conditions or sequences of conditions could perhaps represent less arbitrary disease delineations. Both narratives involve loss of tissue adhesion as a function of changes in the tissue. Interestingly, strategies that target retained tissue dependencies are among the most successful medical approaches in oncology. This comprise oestrogen deprivation therapy by oestrogen antagonists or aromatase inhibitors in hormone receptor positive breast cancer (Harbeck and Gnant 2017) as well as androgen deprivation therapy in prostate cancer (Attard, Parker et al. 2016). This further account for the anti-leukaemic responses induced by FLT3 inhibition in patients who are not FLT3 mutated (Knapper, Burnett et al. 2006, Fischer, Stone et al. 2010).

Precision medicine and the complexity of biological systems

The central dogma in molecular biology is the unidirectional flow of information from genes to proteins; the "genotype-phenotype relationship" (Crick 1958). This bottom-up model has dominated the experimental work of biomedicine as well as the interpretation of observational data. Although the results and arguments put forward in this thesis in no way question the validity of this dogma per se it challenges the application of the scientific approaches founded on the dogma:

the emphasis of understanding the "genotype-phenotype relationship" in linear unidirectional terms. To summarise my argument in general terms: metazoan cell identity, cell state and cell fate is determined by numerous intrinsic AND extrinsic factors. For any given cell the selection of potential cell identities and cell states is intrinsically defined by the cell's genetic material, the DNA. Through quantitative or qualitative alterations of complex gene-interactions a somatic mutation can reshape the trajectories of cell fate. Through emergence of new molecular features mutations in the regulatory part of the DNA or mutated gene products can open up unconventional transcriptional states resulting in novel cellular properties. Cell identity and cell state is further strongly influenced by the cell´s line of descent, essentially defining the cells epigenetic configuration and confining the cells potential differentiation paths. Fundamentally metazoan cells are, however, neither self-sufficient nor self-governing. Metazoan cells are collective in nature, and every new cell develops into being profoundly embedded in context. Networks of cells co-produce and co-maintain tissue and organ integrity, and collectively perform plastic transformations in response to perturbations. Through interactions like physical contact, autocrine, paracrine and endocrine signals, the collective of cells co-operate through continuously modifying their individual epigenomes and transcriptomes, in response to their surroundings. (Bertolaso and Dieli 2017). Under these premises, cancer, although frequently described as a "genetic" disease, more fundamentally is a manifestation of aberrant cell behaviour. Although mutations can change the boundary conditions for a cell's repertoire of potential phenotypic expression the effect of a mutation on a cell is profoundly relational. As a cell or a line of descending cells phenotypically diversify by expressing non-canonical transcriptional states it is in part the conditions of the environment that defines if the change is beneficial or deleterious. The emergence of novel cellular properties can accordingly never be fully understood or accounted for at the cellular or sub-cellular level. The gene-environment provides a dynamic and relational substrate where the meaning of the gene variant is defined. As neoplastic properties emerge by force of gene-gene-environment alterations the most relevant question may not be how mutations arise and translate to change but how the gene gene-environment relationship restrict the potential translational effect of novel gene-variants. Indeed, the nature of the "phenotype – emergent genotype relation" appears as a highly promising field to explore in cancer. Genotype emergence can under these premises be predictive of disease trajectories through association rather than through causation.

From this argument certain implications would seem to follow for the expectations of current precision oncology related approaches in AML.

Possible limitations with regards to molecular delineation of AML and AML subtypes

In AML accurate disease delineation is important as treatment decisions increasingly rely on biomarkers rather than clinical disease presentation. With increasing use of sequencing technologies in otherwise healthy individuals' incidental findings of clonal haematopoiesis and pre-leukaemia are anticipated to rise. Leukaemic transitions seem, at least in some instances, to be multidimensional, challenging the validity of molecular defined disease delineation, as well as early and post-treatment detection of disease, as defined by molecular assays.

The emergence of cell populations characterised by mutations in alternate signalling genes, as well as isoform switching under targeted therapy, suggest plasticity between the pre-leukaemic and leukaemic cell pool. The heterogeneity of the pre-leukaemic pool is not known. If the heterogeneity is extensive enough it could potentially challenge the validity of molecular sub-classification. The perspective of relationality further suggests potential arbitrariness with regards to current sub-classification practises. AML disease stratification is strongly influenced by the ability to separate individuals into various risk groups based on cytogenetic and molecular genetic features (Arber, Orazi et al. 2016). Various subgroups of AML therefore largely reflect variation in response to the treatment combination of anthracyclines and cytarabine. It is not clear from a biological perspective how variation in specific treatment response reflects disease causality. Notably, several of the poor risk groups are associated with previous exposure to genotoxic treatment which potentially could account for their poor treatment outcome. In biological terms, one may hypothesise that these cells were already selected for by their ability of sustaining genotoxic stress without induction of apoptosis. It is not clear if genetic features would always be the best predictor of variation in treatment response. Subgroup analysis following a different treatment regimen would perhaps result in different separation. One could speculate whether drugs specifically targeting leukaemic stem cells or differentiation agents would lead to stronger predictive signals with regards to gene expression patterns or cytomorphology. Indeed, patients characterised by poor risk factors have been shown to respond comparably favourable to decitabine treatment (Welch, Petti et al. 2016).

Molecular targeted therapy and opportunities and challenges in AML

Plurality of genotypically and phenotypically diverse cell populations characterised by discrete properties challenge the potential of molecular targeted therapy. Even if leukaemic transitions are poly-clonal several observations support a shared genetic ancestry in most cases (Ding, Ley et al. 2012, Dvorakova, Racil et al. 2013, Jan and Majeti 2013, Hirsch, Zhang et al. 2016, Cocciardi, Dolnik et al. 2019). To what extent collective genetic identities represent therapeutic opportunities remains undetermined. The potential is supported by the benefit of all-trans-retinoic acid treatment in acute promyelocytic leukaemia, directed at what is considered an early "hit". What may limit

the benefit of this strategy is, however, phenotypic heterogeneity as well as plasticity within a genotypically defined cell population. Variable expression and function of mutated gene products across the functional space of cancer cells may limit the efficacy of any molecular targeted strategy. As with *FLT3*-LMs, the variant expression or lack of expression on cells with self-renewal capabilities or potential for induction of self-renewal capabilities is non-trivial. If *FLT3*-LMs occur in leukaemic stem cells that do not express the gene and the impact of the mutation only presents as the cells progeny gradually differentiate then FLT3 targeted therapy will likely be of little utility in the eradication of the leukaemic stem cells. Analogously leukaemic stem cells in chronic myeloid leukaemia do not seem to depend on the BCR-ABL fusion proteins, perhaps accounting for why imatinib treatments does not cure chronic myeloid leukaemia (Hamilton, Helgason et al. 2012).

Targeted therapy confers a highly stringent constraint not only on target cancer cells but also all other cells expressing the target molecule. Since the properties of any cellular component are functions of its interactions it seems fair to assume that inhibition of any molecule will have context-specific consequences. Targeted therapy may therefore mediate clinical effects through mechanisms not considered when rationally developing the treatment, as well as result in unexpected toxicities and long-term consequences. *In vitro* tyrosine kinase inhibitor treatment of *FLT3*-LM cells is characterised by emergence of secondary mutations conferring therapy resistance. The mutational pattern has further been shown to differ in accordance with specific agents (von Bubnoff, Engh et al. 2009), and include mutations in other genes leading to activation of parallel signalling pathways, including *NRAS* variants (Piloto, Wright et al. 2007). The conceptualisation and categorisation of *FLT3*-LM as a distinct disease entity (in practical rather than formal terms), and the subsequent development and application of FLT3 targeted therapies have contributed in shaping novel AML disease trajectories characterised by the emergence of novel gene variants as well as unusual combinations of mutated genes, including the co-occurrence of *FLT3*-LMs and *NRAS* mutations (McMahon, Ferng et al. 2019, Zhang, Savage et al. 2019). One could imagine that changes in therapeutic practices could result in altered AML demographics where the composition of molecular characterised categories change reflecting novel constraints. While "the expectation is that progress in developing targeted therapies with reduced nonspecific cytotoxicity will result in a decrease in t-MN [therapy-related myeloid neoplasm] incidence" (McNerney, Godley et al. 2017), one could speculate whether targeted therapies confer an even stronger selective constraint on haematopoietic cells with an intrinsic but contained malignant potential, and that this potential could be unleashed through targeted treatment for unrelated conditions. Up to 16 different *FLT3*-LMs have been reported in single individuals at AML presentation and could indicate that *FLT3*-LM are frequent molecular events, that under "normal" condition do not result in emergence (Blatte, Schmalbrock et al. 2019). One could hypothesise that

off-target FLT3 inhibition as a result of broad tyrosine kinase inhibitors could result in expansion of *FLT3* mutated bone marrow cells. While supported by the development of *NRAS* mutated neoplasm following BRAF inhibition a consistent increase in secondary malignancies in chronic myeloid leukaemia patients treated with imatinib has not been observed (Miranda, Lauseker et al. 2016). Currently targeted therapies are predominantly administered in an end-of life setting. It will be important to monitor if the use of molecular targeted therapies up front, in adjuvant or consolidation treatment regiments, will result in emergence of neoplastic properties and novel forms of treatment related neoplasms. This is of particular importance considering therapeutic intervention in a preventive setting.

Knowledge, predictions and action in AML

Risk assessment is a core approach in precision oncology. Successful risk-adapted therapy implies identification of risk followed by a mitigating intervention that improves the outcome of interest. This activity presupposes knowledge of the risk distribution as well as knowledge and availability of efficient interventions. The added benefit of this strategy can be asserted by comparing outcome in individuals that were treated in accordance with this strategy and those that were not. Such comparisons are preferably performed prospectively in randomised clinical trials, but such studies are often not performed. Risk-adapted management instead frequently rely on identification of risk followed by treatment modification where the added benefit is deduced by subgroup analysis or, in some cases remains hypothesised.

In AML risk-adapted therapy based on cytogenetic and molecular profiling as well as therapeutic response assessment is recommended. The added benefit of many of these recommendations are, however, poorly validated and quantified (Rashidi, Walter et al. 2016, Dohner, Estey et al. 2017). The poor outcome associated with *FLT3*-LMs early on led to recommendations of allogeneic haematopoietic stem cell transplantation in first complete remission. Although observational studies suggest that this practice has result in improved outcomes prospective trials asserting the value of this intervention are missing (Dohner, Estey et al. 2010, Dohner, Estey et al. 2017). Analogously, despite lack of randomised clinical trial results demonstrating an added benefit of sorafenib in *FLT3*-LM AML (Serve, Krug et al. 2013) the drug has in some centres been administered in addition to standard chemotherapy in *FLT3*-LM patients since the beginning of the 2000s. Recent retrospective observational reports suggest that this has provided benefit (Sasaki, Kantarjian et al. 2019, Yalniz, Abou Dalle et al. 2019), but the study designs cannot provide definite answers. Similarly, FLT3-targeted maintenance therapy in a post-transplant setting has been integrated in clinical care before randomised trials were performed. Retrospective assessment demonstrates correlations between sorafenib treatment and improved outcomes (Brunner, Li et al.

2016, Xuan, Wang et al. 2018, Battipaglia, Massoud et al. 2019), but solid conclusions cannot be drawn. Similarly, sorafenib treatment following relapse after allogeneic stem cell transplantation has been indicated to provide benefit as a salvage regimen (Xuan, Wang et al. 2019). While minimal residual disease assessment has received increased attention in the management of AML, evidence supporting the hypothesis that minimal residual disease informed clinical management significantly improve outcome in AML currently remain scares (Ommen 2016).

Predictions of risk are by their nature probabilistic as they are inferred from evidence at a group level. Furthermore, the accuracy of risk estimates has to be evaluated at the group level, as the event rate for any given individual is either 1 or 0. Disease stratification results in smaller sample size. Over time entire sample populations change in line with evolving therapeutic landscapes. The influence treatment practices have on cell and disease trajectories suggest that one cannot transfer knowledge of predictive relationships across treatment regimens (Welch, Petti et al. 2016). As an example, an added benefit of sorafenib as post-transplant maintenance treatment may be standard-induction-therapy specific. An induction-regimen comprising midostaurin maintenance, as is now frequently recommended in *FLT3*-LM AML, could lead to a residual cell fraction that does not respond to sorafenib. Similarly, as induction regimens diversify most likely so will the molecular composition of relapsed and refractory AML. It is not obvious that evidence of comparative effectiveness derived from this group will remain valid as the sample composition change in accordance with novel treatment strategies. Paradoxically precision medicine related approaches may result in decreased availability of knowledge that can inform clinical decision making, thus reducing the predictive precision with regards to benefits and hazards related to single interventions.

The imaginary of precision medicine and unintended consequences

There is no sharp demarcation between precision and non-precision related medical approaches is challenging as precision oncology in many regards is a continuation of the reductionist biomedical traditions characterising medicine in the 20th and 21st century. As such, in the introduction I emphasised that the delineation of precision medicine is not best understood as evolution of practices, but rather as an expansion from medical practice to a techno-scientific imaginary. Although precision medicine has been presented as a societal endeavour, it is not the destination but rather a technical solution to a political oriented objective: namely to improve public health (Figure 18). Precision oncology is as such a means to this end and not a goal in and of its self. The question is therefore not if precision oncology is feasible, but if it is “feasible enough”, as to be both desirable, viable and sustainable within certain frames. This is a combined scientific, medical, political, economic and ethical question. As a medical, political, economic, and societal aim the

intended and unintended consequences related to precision medicine far exceed the sum of measurable effects of single medical interventions. The hope, vision and objective of precision medicine shape research objectives, policy agendas (Horgan, Lawler et al. 2015), legal and regulatory frameworks, health care delivery systems and public expectations across the world (Tarkkala, Helén et al. 2019).

In a well cited AML review paper Ferrara and Schiffer summarise the molecular knowledgebase with regards to AML pathophysiology and conclude: “Hopefully, this new biological information will contribute to less empirical approaches to treatment” (Ferrara and Schiffer 2013). This statement embodies seemingly prevailing thinking in the field. Precision medicine promotes a substantial change in the foundation of clinical decision making, characterised by increased reliance on bio-plausibility and de-evaluation of evidence. This is reflected in oncological practice. Stakeholders of precision oncology advocate increased pace in the translation and implementation of novel “promising” agents. This has resulted in deregulation and reduced evidence requirements for marked authorisation of novel drugs, including increased reliance on single arm studies as well as poorly validated surrogate endpoints (Chen, Raghunathan et al. 2019, Gyawali, Hey et al. 2019, Hilal, Sonbol et al. 2019, Zettler, Basch et al. 2019). Based on a systematic evaluation of cancer drugs approved by the European Medicines Agency in the period 2009-2013, Davies et al. demonstrate that the majority of novel oncological agents were approved with no clear evidence of clinical benefit and that evidence of clinically meaningful utility remained unfounded a minimum of 3 years after approval. Quantifiable benefits were marginal, and the estimated median life expansion provided when documented was only 2.7 months (Range:1 – 5.8 months) (Davis, Naci et al. 2017). Early marked-approval is further dis-incentivising for execution of confirmatory well powered randomised studies, resulting in absent quantification of comparative effectiveness. Lack of high level evidence further affect the possibility and validity of cost-benefit analysis (DeLoughery and Prasad 2018). Low-grade evidence paradoxically increase uncertainty in clinical decision making (Moscow, Fojo et al. 2018). Despite the failing empirical foundation (Djulbegovic and Ioannidis 2019) uptake of low-grade evidence in clinical care is substantial. There is an increase in use of off-label targeted therapy (Saiyed, Ong et al. 2017), despite evidence suggesting inefficiency (Le Tourneau, Delord et al. 2015). Some countries and health care delivery systems even have aliquoted funds for such practises, like the National Health Service (NHS, UK) Cancer Drug Funds. A recent analysis of the use of such solutions suggest no meaningful societal or patient value gain (Aggarwal, Fojo et al. 2017).

These sobering results suggest that there is an increasing distance between the expectations and realisations of precision oncology. Repercussions of this friction are currently materialising across a wide range of medical as well as social domains (Fojo, Mailankody et al. 2014, Bowen and

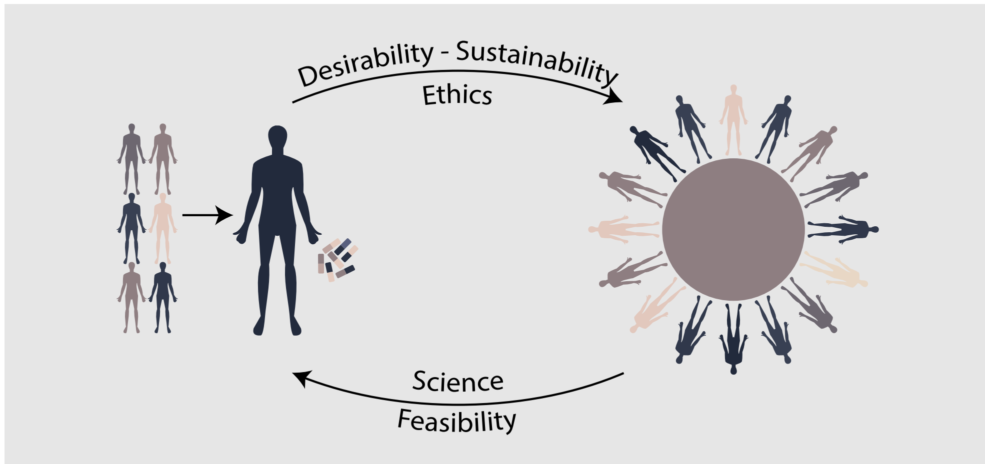

Figure 18. Precision medicine is a techno-scientific solution to a political problem.

Casadevall 2015, MacLeod, Harris et al. 2016). The gradual implementation of low value precision oncology related strategies has contributed to a situation where the total financial burden of cancer treatment and cancer care is rapidly spiralling out of control (Aggarwal, Ginsburg et al. 2014). This has resulted in significant financial toxicity for cancer patients (Knight, Deal et al. 2018). In settings characterised by resource constraint this has further generated restrictions in the priority setting, which ultimately result in reduced availability of novel therapeutic agents within the frames of both public healthcare systems as well as from insurance providers. With these agents being available in the free market an increasing discrepancy in access to care is materialising. Individual cancer patients no longer only fight their disease, they also battle public institutions or insurers for access to treatment (Aggarwal, Ginsburg et al. 2014). Desperation, fear and increasing inequity may negatively influence phenomenological aspects of living and dying from cancer. Such experiences may further contribute in shaping public discourse, conceivably resulting in justified erosion of trust in scientific knowledge, medicine and policy makers.

Future perspectives

This work has focused on a narrow selection of the AML literature as well as the knowledge base of *FLT3*-LM AML. By restricting the analysis in such a way, it is possible that I have captured only a limited range of assumptions and practices related to precision haemato-oncology. Despite these limitations I do believe that the considerations put forward in the discussion are relevant to other cancer related disciplines, as they refer to general reflections regarding the nature of cancer cells and how this relates to predominating theories of cancer as well as oncological knowledge production and clinical practice.

The existence of limitations and disadvantages, in our case of somatic mutation theory and precision oncology, does not imply that they are without benefits. From a practitioner's perspective the quality of a theory or a practice is not a direct function of its truth value but rather of its fitness for purpose (Funtowicz and Ravetz 1993). Experience has demonstrated that medical strategies based on somatic mutation theory are feasible, despite inherent biological limitations. Expansion of cell populations characterised by certain molecular markers may correlate with disease trajectories, and disease stratification and risk-adapted management as well as molecular targeted therapy does sometimes transform outcome in cancer subgroups. Combination regiments or sequential administration of drugs targeting various molecular pathways may ultimately improve response rates and temporally overcome resistance. Longitudinal disease monitoring and response assessment may further improve treatment algorithms, eventually resulting in improved outcome. Functional assessment like drug sensitivity and resistance testing or single cell signalling profiles may outperform molecular profiling as predictors of therapeutic response. In line with this reasoning what remains important is quantification of utility. I would argue that with regards to precision oncology utility must not only be demonstrated with regards to therapies but also in relation to methodology, and I would further argue that there is potential for strengthening the empirical evidence base with regards to the utility of current practices.

The interpretation of *FLT3*-LM AML put forward in this thesis is intended to generate novel questions related to the biology of cancer and how it can be therapeutically exploited. In particular, I have wished to open up questions related to the relative contribution of somatic diversification, selection, adaptation and contingency in oncogenesis in general. Resolution of these questions is not trivial and could inform the expectations in the present regarding the potential utility of both gene and cell centred screening and treatment monitoring approaches, in addition to the potential value of targeting specific molecular features. It could further inform the direction of research initiatives intended at reducing the risk of neoplastic progression. Limitations associated with the current gene and cell-centred paradigm further suggest that expanding perspectives may open up novel ways of conceptualising, categorising, and treating cancer that may advance the field.

Conclusions

This thesis has tried to explore an instance of the relationship between disease theory and precision medicine, in the highly specialised case of *FLT3*-LM AML. The current politico-medical discourse of precision oncology is largely built on theories and visions rather than realisations. Identification of incoherent explanatory models as well as arbitrariness in current practices indicates fragility in the relationship between knowledge and theory, undermining the legitimacy of current theoretically based practices. Disease dynamics, relational properties and casual contribution from several levels

of biological organisation comes into conflict with the linear monocausal explanatory model on which precision oncology is largely built. Moreover, it suggests limitations with regards to the precision obtainable in molecular categorisation, precise predictions, and the idea of categorical "molecular targets". The public discourse suggests that substantial progress is anticipated to result from continued pursuit of precision oncology. This friction calls for careful thinking and informed reasoning on the topic of what precision oncology in its current form can and cannot be expected to deliver, both in science, medicine and society. For this explicit purpose I believe that conceptual frameworks founded on somatic mutation theory are inadequate. Further, the translational emphasis characterising precision medicine related research and the gradual shift from evidence to theory-based practices suggest that the imaginary of precision medicine contributes to the maintenance and validation of the theoretical frameworks on which the imaginary is built. In particular, I believe that a word of caution is needed in the presence of strong incentives to translate medical knowledge ever more and ever faster. Scientific and medical progress in the era of precision oncology requires epistemic, clinical, political and social awareness.

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Supplementary material

Supplementary table 1 – PMID result from literature search

19372391	20385793	22265402	15687234	26095251	21220598	16435386	12086889	23591789
23634996	16532500	20026804	14701873	21575865	25487151	20733134	14966562	10698507
21130701	12011120	22898539	15146183	10860979	16921040	19638619	16109776	21118985
19880497	16998484	12717436	18395287	15345597	19570512	12646957	19417208	21494260
19657110	17126723	17283117	18308931	14966519	12970773	11222358	20212254	28644114
21251613	22375970	15084693	20038799	19654408	15753458	22737091	24622513	12518367
18468541	27276561	20142433	21220605	22158538	20664057	11090077	27526324	16397263
21814200	12036858	18187662	12951584	12897778	10675114	17315155	12200686	17008539
22237025	24522528	11290608	18955566	12393388	11585760	12393424	22086414	17942707
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17126425	19632179	14562125	21385853	14726387	17957027	17996649	11836210	23399072
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21067377	17041095	16868251	16051734	21892162	22062686	16921041	12194988	11796918
19668202	20305640	17045204	21251617	22464800	11960327	18230792	22094260	15644454
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19474426	11110676	17714993	15920007	18337557	15226186	19171880	19056693	11526243
21909114	15931389	19898489	19388938	19430464	16014563	10753120	20451454	19218430
22417203	19211935	19776406	21045145	21178137	21892158	15619633	11410481	18840713
18987736	21873988	15170211	17962510	20428194	16455956	17589499	19262598	20631300
22817890	12176867	20368543	12563308	16882711	12414645	16857985	19652201	20692206
15632206	18299402	21598255	16778214	18945962	22057347	18809607	11756186	21177505
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25426838	14764878	21220611	18450603	12971829	24429522	22308295	24478400	20008787
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18450602	18299451	11242107	15870183	19401558	21270442	20507306	16899611	14759363
22740453	17952057	21803851	17371948	16960150	24703711	20026803	18832655	16082245
21949397	21399634	22504184	20581313	22897849	19282459	20212066	20705759	17515897
22375971	22417201	11588023	15156177	22482940	18538732	15365188	22279045	18716133
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15659725	20339075	18977325	17589498	15104525	17179228	20385359	16109713	25987659
11731795	17251531	20567020	19850741	10815900	11090046	21474104	20053758	
21714648	16455952	12531805	11021753	20368434	21172891	17804695	20160717	
15084694	27895058	23333149	18832181	15010150	12839953	20029046	23063124	
12393746	15260991	14604977	19776405	16885047	12623843	20616797	25550361	

Supplementary table 2 – WHO classification system

2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia
Acute myeloid leukemia (AML) and related neoplasms

AML with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22.1);RUNX1-RUNX1T1
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11
- APL with PML-RARA
- AML with t(9;11)(p21.3;q23.3);MLLT3-KMT2A
- AML with t(6;9)(p23;q34.1);DEK-NUP214
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RBM15-MKL1
- Provisional entity: AML with BCR-ABL1
- AML with mutated NPM1
- AML with biallelic mutations of CEBPA
- Provisional entity: AML with mutated RUNX1

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasms

AML, not otherwise specified
- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic/monocytic leukemia
- Pure erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome
- Transient abnormal myelopoiesis (TAM)
- Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

Acute leukemias of ambiguous lineage
Acute undifferentiated leukemia
MPAL with t(9;22)(q34.1;q11.2); BCR-ABL1** MPAL with t(v;11q23.3); KMT2A rearranged MPAL, B/myeloid, NOS
MPAL, T/myeloid, NOS

Adaptet from Arber et al and Dohner et al. (Arber, Orazi et al. 2016, Dohner, Estey et al. 2017)

Supplementary table 3 – ELN risk stratification system

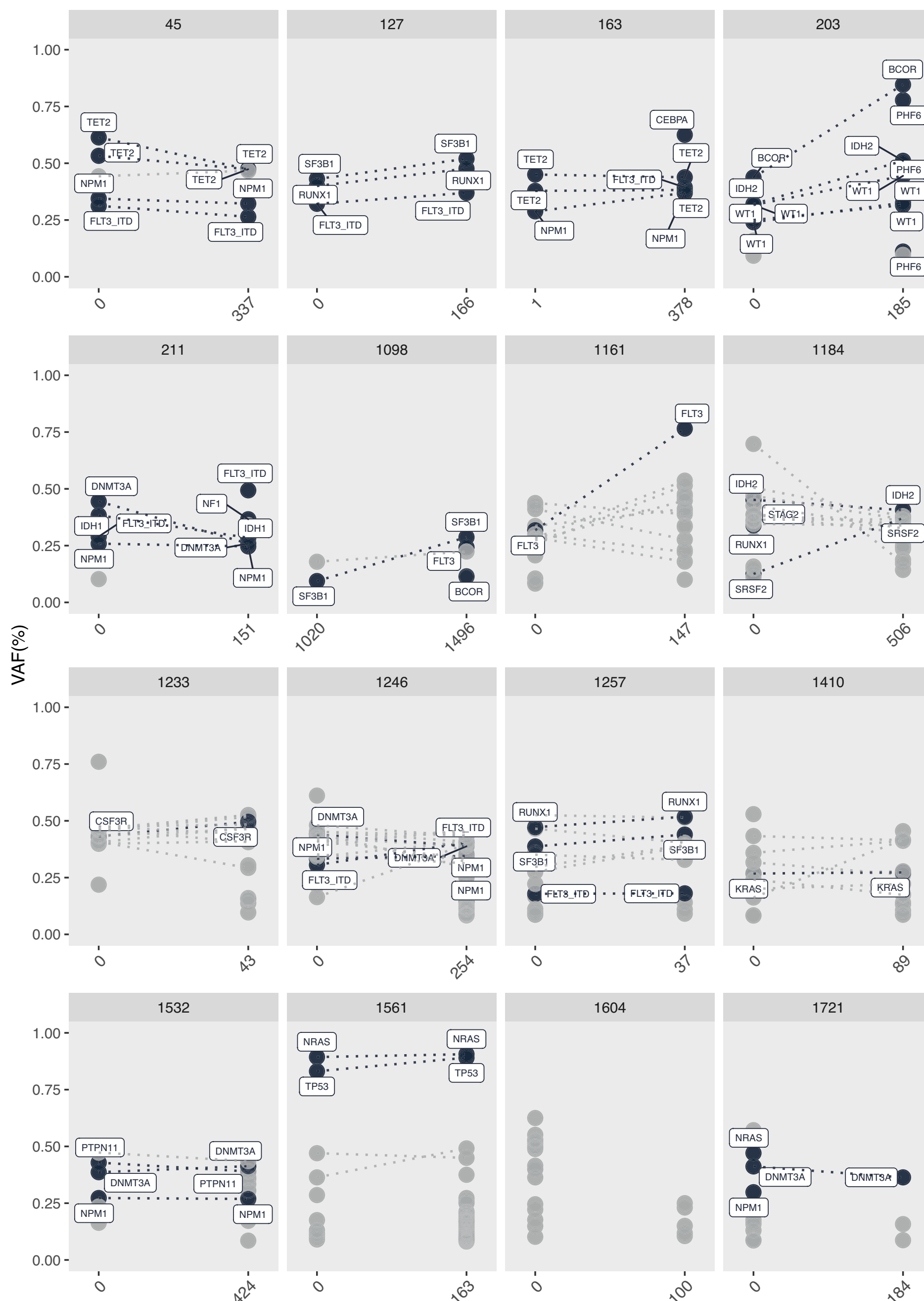
2017 ELN risk stratification by genetics (Dohner, Estey et al. 2017)	
Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1) - RUNX1-RUNX1T1 inv(16)(p13.1q22) or t(16;16)(p13.1;q22) - CBFB-MYH11 Mutated NPM1 without FLT3-ITD or with FLT3-ITD^{low}[1] Biallelic mutated CEBPA
Intermediate	Mutated NPM1 and FLT3-ITD^{high}[1] Wild-type NPM1 without FLT3-ITD or with FLT3-ITDl^{ow}[1, 7] t(9;11)(p21.3;q23.3) - MLLT3-KMT2A[2] Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1) - DEK-NUP214 t(v;11q23.3) - KMT2A rearranged t(9;22)(q34.1;q11.2) - BCR-ABL1 inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) - GATA2,MECOM(EVI1) 25 or del(5q); 27; 217/abn(17p) Complex karyotype[3] Monosomal karyotype[4] Wild-type NPM1 and FLT3-ITD^{high}[1] Mutated RUNX1[5] Mutated ASXL1[5] Mutated TP53[6]

Frequencies, response rates, and outcome measures should be reported by risk category, and, if sufficient numbers are available, by specific genetic lesions indicated.
*Prognostic impact of a marker is treatment-dependent and may change with new therapies.
1. Low, low allelic ratio (<0.5); high, high allelic ratio (</=0.5); semiquantitative assessment of FLT3-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve "FLT3-ITD" divided by area under the curve "FLT3- wild type"; recent studies indicate that AML with NPM1 mutation and FLT3-ITD low allelic ratio may also have a more favorable prognosis and patients should not routinely be assigned to allogeneic HCT.
2. The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations.
3.Three or more unrelated chromosome abnormalities in the absence of 1 of the WHO-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with BCR-ABL1.
4. Defined by the presence of 1 single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).
5. These markers should not be used as an adverse prognostic marker if they co- occur with favorable-risk AML subtypes.
6. TP53 mutations are significantly associated with AML with complex and monosomal karyotype.
7. When without adverse-risk genetic lesions

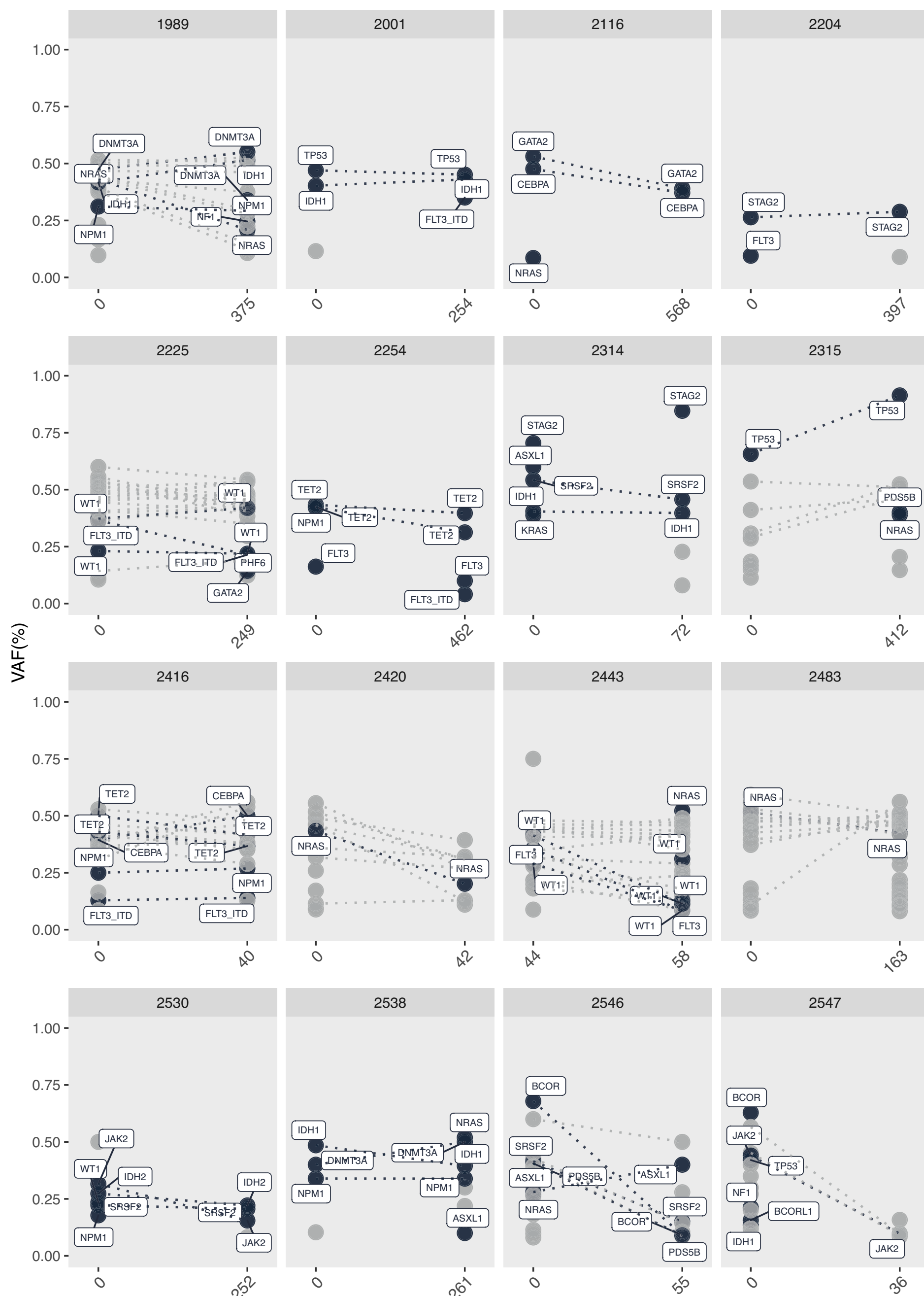
Adaptet from Dohner et al. (Dohner, Estey et al. 2017)

Supplementary figure 1 – Serial sampling and exome sequencing in Beat-AML

Patient ID	Sex	Age	Fusion	Cumulative Treatment Types	Cumulative Treatment Regimens	Vital Status	Overall Survival	Cause Of Death
17	Female	27	None	Standard Chemotherapy, Bone Marrow Transplant, Targeted Therapy - Kinase Inhibitor(s)	7+3 (Cytarabine, Idarubicin), HiDAC, Fludarabine, TBI, Sorafenib, Hydroxyurea, Decitabine	Dead	1463	Dead-Disease
45	Female	70	None	Standard Chemotherapy, Intrathecal	7+3 (Cytarabine, Idarubicin), MiDAC, Cytarabine, Methotrexate	Dead	511	Dead-Other
127	Male	74	None	Standard Chemotherapy, Supportive/Palliative Care, Targeted Therapy - Kinase Inhibitor(s)	Decitabine, Quizartinib, Mitoxantrone	Dead	1264	Dead-Unknown
163	Male	73	None	Standard Chemotherapy, Targeted Therapy - Kinase Inhibitor(s)	7+3 (Cytarabine, Idarubicin), MiDAC, AC220 (Ambit)	Dead	425	Dead-Disease
174	Male	69	None	Standard Chemotherapy, Targeted Therapy - Kinase Inhibitor(s)	7+3 (Cytarabine, Idarubicin), MiDAC, Hydroxyurea, AC220 (Ambit)	Dead	419	Dead-Disease
191	Female	60	None	Standard Chemotherapy, Supportive/Palliative Care, Targeted Therapy - Kinase Inhibitor(s)	7+3 (Cytarabine, Idarubicin), HiDAC, MEC (Cytarabine, Etoposide, Mitoxantrone), Etoposide, Mitoxantrone, Quizartinib	Dead	394	Dead-Disease
203	Male	70	None	Standard Chemotherapy, Targeted Therapy - Kinase Inhibitor(s)	7+3 (Cytarabine, Idarubicin), HiDAC, Quizartinib, Idarubicin and Cytarabine	Dead	400	Dead-Disease
211	Female	65	None	Standard Chemotherapy, Bone Marrow Transplant, Intrathecal, Targeted Therapy - Kinase Inhibitor(s)	Fludarabine, Melphalan, 7+3 (Cytarabine, Idarubicin), MiDAC, Quizartinib, Methotrexate	Dead	387	Dead-Unknown
652	Male	71	None	Standard Chemotherapy, Supportive/Palliative Care, Targeted Therapy - Kinase Inhibitor(s)	5+2 (Cytarabine, Idarubicin), Dasatinib, 7+3 (Cytarabine, Idarubicin), Azacitidine, Hydroxyurea	Dead	309	Dead-Disease
1098	Female	31	None	Standard Chemotherapy, Bone Marrow Transplant, Targeted Therapy - Other, Targeted Therapy - Kinase Inhibitor(s)	Fludarabine, Melphalan, Dasatinib, 7+3 (Cytarabine, Idarubicin), HiDAC, FLAG-IDA (Cytarabine, Filgrastim, Fludarabine, Idarubicin), Azacitidine, MEC (Cytarabine, Etoposide, Mitoxantrone), Rituximab, Busulfan, Cyclophosphamide	Dead	2661	Dead-Unknown
1161	Female	30	MLLT3-KMT2A	Standard Chemotherapy, Supportive/Palliative Care	GCLAM (G-CSF, Cladribine, Cytarabine, and Mitoxantrone) , 7+3 (Cytarabine, Idarubicin), HiDAC, Decitabine	Dead	270	Dead-Disease
1184	Male	52	None	Standard Chemotherapy, Bone Marrow Transplant, Targeted Therapy - Other	7+3 (Cytarabine, Idarubicin), HiDAC, AG-221, HAM (Mitoxantrone, Cytarabine) plus Crenolanib, Fludarabine, Melphalan, ATG	Dead	1237	Dead-Disease
1233	Male	61	None	Standard Chemotherapy	5+2 (Cytarabine, Idarubicin), 7+3 (Cytarabine, Idarubicin), MEC (Cytarabine, Etoposide, Mitoxantrone), Decitabine	Dead	370	Dead-Disease
1246	Male	62	None	Standard Chemotherapy, Bone Marrow Transplant, Targeted Therapy - Kinase Inhibitor(s)	7+3 (Cytarabine, Idarubicin), HiDAC, FLAG-IDA (Cytarabine, Filgrastim, Fludarabine, Idarubicin), Crenolanib, Clofarabine, Cytarabine, Busulfan, Cyclophosphamide	Dead	568	Dead-Unknown
1257	Male	73	None	Standard Chemotherapy, Targeted Therapy - Kinase Inhibitor(s)	Cytarabine, Fludarabine, Sorafenib, Crenolanib, Azacitidine, Sorafenib, Decitabine, Sorafenib , Clofarabine, Cytarabine, Sapacitabine (CYC682-06)	Dead	658	Dead-Unknown
1410	Male	38	GATA2-MECOM	Standard Chemotherapy, Supportive/Palliative Care	7+3 (Cytarabine, Idarubicin), FLAG-IDA (Cytarabine, Filgrastim, Fludarabine, Idarubicin), Azacitidine, MEC (Cytarabine, Etoposide, Mitoxantrone)	Unknown	Missing	Missing
1532	Female	46	None	Standard Chemotherapy	7+3 (Cytarabine, Idarubicin), Azacitidine, MEC (Cytarabine, Etoposide, Mitoxantrone), MiDAC	Alive	788	Alive
1561	Male	23	None	Standard Chemotherapy, Targeted Therapy - Kinase Inhibitor(s)	Trametinib, HAEP (Cytarabine, Etoposide, Cisplatin)	Dead	318	Dead-Disease
1604	Male	58	CBFB-MYH11	Standard Chemotherapy	7+3 (Cytarabine, Idarubicin), HiDAC, ODSH (2 O, 3-O Desulfated Heparin)	Alive	761	Alive
1721	Male	68	None	Standard Chemotherapy	7+3 (Cytarabine, Idarubicin), HiDAC, Azacitidine, MEC (Cytarabine, Etoposide, Mitoxantrone)	Alive	976	Alive
1727	Male	45	None	Standard Chemotherapy, Supportive/Palliative Care	GCLAM (G-CSF, Cladribine, Cytarabine, and Mitoxantrone) , 7+3 (Cytarabine, Idarubicin), Azacitidine, MEC (Cytarabine, Etoposide, Mitoxantrone), Decitabine	Dead	259	Dead-Disease
1973	Male	70	None	Standard Chemotherapy, Supportive/Palliative Care	7+3 (Cytarabine, Idarubicin), Azacitidine, Decitabine	Alive	523	Alive
1989	Male	46	None	Standard Chemotherapy	HiDAC, GCLAC (GCSF, Clofarabine & Cytarabine), SWOG S1203 (7+3 +/- Vorinostat), MEC, Lenalidomide, Etoposide, Cytarabine	Unknown	Missing	Missing
2001	Female	67	None	Standard Chemotherapy, Supportive/Palliative Care, Targeted Therapy - Kinase Inhibitor(s), Targeted Therapy - Other	AG-120, MEC (Cytarabine, Etoposide, Mitoxantrone), Sorafenib, Hydroxyurea	Dead	449	Dead-Disease
2116	Female	10	None	Standard Chemotherapy	COG-AAML1031 (IT/IV Cytarabine, Daunorubicin, Etoposide, Mitoxantrone +/- Bortezomib or Sorafenib), ADE (Cytarabine, Daunorubicin & Etoposide), FLAG (Fludarabine, High-dose Cytarabine, G-CSF)	Alive	569	Alive
2119	Male	76	None	Standard Chemotherapy	CPX-351 (Liposomal Cytarabine + Daunorubicin), Decitabine, Incyte trial (azacitidine and JAK 1 inhibitor)	Dead	520	Dead-Unknown
2204	Female	2	None	Standard Chemotherapy, Bone Marrow Transplant, Intrathecal	COG-AAML1031 (IT/IV Cytarabine, Daunorubicin, Etoposide, Mitoxantrone +/- Bortezomib or Sorafenib), Cytarabine, FLAG (Fludarabine, High-dose Cytarabine, G-CSF), Busulfan, Cyclophosphamide	Alive	428	Alive
2225	Female	71	None	Standard Chemotherapy, Supportive/Palliative Care	7+3 (Cytarabine, Idarubicin), Azacitidine, CLAG-M (Cladribine, Cytarabine, Mitoxantrone, Filgrastim), Hydroxyurea, Mitoxantrone	Dead	280	Dead-Disease
2254	Female	52	None	Standard Chemotherapy	7+3 (Cytarabine, Idarubicin), HiDAC, MEC (Cytarabine, Etoposide, Mitoxantrone)	Dead	633	Dead-Disease
2314	Male	68	None	Standard Chemotherapy	3+7 (Cytarabine, Daunorubicin)	Dead	122	Dead-Unknown
2315	Male	62	None	Standard Chemotherapy	Decitabine, Hydroxyurea	Dead	424	Dead-Disease
2337	Male	53	None	Standard Chemotherapy, Targeted Therapy - Kinase Inhibitor(s)	HAM (Cytarabine, Mitoxantrone), 3+7 (Cytarabine, Daunorubicin), Sorafenib, Hydroxyurea	Dead	168	Dead-Disease
2416	Female	72	None	Standard Chemotherapy, Targeted Therapy - Kinase Inhibitor(s)	7+3 (Cytarabine, Idarubicin), Sorafenib, Ponatinib	Dead	118	Dead-Disease
2420	Male	24	None	Standard Chemotherapy, Bone Marrow Transplant	7+3 (Cytarabine, Idarubicin), HAM (Cytarabine, Mitoxantrone), Azacitidine, Decitabine, Busulfan, Cyclophosphamide	Alive	187	Alive
2443	Female	63	None	Standard Chemotherapy, Supportive/Palliative Care, Targeted Therapy - Other	7+3 (Cytarabine, Idarubicin), Cytarabine, Azacitidine, Sorafenib, Decitabine, Hydroxyurea	Dead	348	Dead-Disease
2477	Male	50	None	Standard Chemotherapy, Bone Marrow Transplant	Busulfan, Fludarabine, 7+3 (Cytarabine, Idarubicin), HiDAC	Alive	576	Alive
2483	Female	46	None	Standard Chemotherapy, Bone Marrow Transplant	7+3 (Cytarabine, Idarubicin), HAM (Cytarabine, Mitoxantrone), Azacitidine, Hydroxyurea, Decitabine, ME (Etoposide, Mitoxantrone), Fludarabine, Melphalan, ATG	Dead	637	Dead-Disease
2530	Female	73	None	Standard Chemotherapy	Ruxolitinib	Dead	560	Dead-Unknown
2538	Male	67	None	Standard Chemotherapy, Targeted Therapy - Other	AG-120, 7+3 (Cytarabine, Idarubicin), FLAG-IDA (Cytarabine, Filgrastim, Fludarabine, Idarubicin), MiDAC	Alive	528	Alive
2546	Male	67	None	Standard Chemotherapy	Vidaza, Cytarabine, 3+7 (Cytarabine, Daunorubicin), Decitabine	Alive	112	Alive
2547	Male	67	None	Standard Chemotherapy	3+7 (Cytarabine, Daunorubicin)	Unknown	Missing	Missing
2611	Female	56	None	Standard Chemotherapy, Bone Marrow Transplant	7+3 (Cytarabine, Idarubicin), HiDAC, GCLAC (GCSF, Clofarabine & Cytarabine), Fludarabine, Cyclophosphamide, Thiotepa, TBI	Dead	323	Dead-Disease
2612	Male	71	None	Missing	Missing	Alive	402	Alive
2654	Female	64	None	Supportive/Palliative Care, Other	Radiation, Paclitaxel, Doxorubicin, Cisplatin, 5-FU	Alive	448	Alive
2685	Male	59	None	Standard Chemotherapy, Bone Marrow Transplant, Targeted Therapy - Kinase Inhibitor(s)	Fludarabine, Melphalan, Azacitidine, Sorafenib	Dead	72	Dead-Disease
2687	Female	61	None	Standard Chemotherapy, Targeted Therapy - Kinase Inhibitor(s)	7+3 (Cytarabine, Idarubicin), HiDAC, FLAG-IDA (Cytarabine, Filgrastim, Fludarabine, Idarubicin), Sorafenib, Hydroxyurea	Dead	263	Dead-Disease
2694	Male	50	RUNX1-RUNX1T1	Standard Chemotherapy	HiDAC, 3+7 (Cytarabine, Daunorubicin)	Alive	424	Alive
2695	Male	49	None	DLI, Standard Chemotherapy, Bone Marrow Transplant, Supportive/Palliative Care	HiDAC, Donor Lymphocyte Infusion, FLAG-IDA (Cytarabine, Filgrastim, Fludarabine, Idarubicin), SWOG S1203 (7+3 +/- Vorinostat), Hydroxyurea, Decitabine, Bu/Flu/TBI (Busulfan, Fludarabine, Total Body Irradiation)	Alive	833	Alive
2704	Female	29	None	Standard Chemotherapy, Bone Marrow Transplant, Supportive/Palliative Care	FLAG (Fludarabine, High-dose Cytarabine, G-CSF), Hydroxyurea, Decitabine, Fludarabine, Cyclophosphamide, TBI , Trametinib	Dead	129	Dead-Disease
2739	Male	66	None	Standard Chemotherapy	Hydroxyurea	Alive	189	Alive
2747	Male	62	None	Standard Chemotherapy, Bone Marrow Transplant	Azacitidine, Fludarabine, Melphalan, ATG	Alive	256	Alive
3990	Female	69	None	Standard Chemotherapy, Targeted Therapy - Kinase Inhibitor(s), Targeted Therapy - Other	7+3 (Cytarabine, Idarubicin), Azacitidine, Midostaurin, Sorafenib, MEC (Cytarabine, Etoposide, Mitoxantrone), MiDAC, Fludarabine, Cyclophosphamide, Thiotepa, TBI, ALRN-6924	Alive	442	Alive
4006	Male	81	None	Supportive/Palliative Care	Vidaza, Decitabine	Dead	97	Dead-Disease
4028	Female	61	None	Standard Chemotherapy, Bone Marrow Transplant	Fludarabine, Melphalan, MiDAC, 7+3 (Cytarabine, Idarubicin) plus Sorafenib	Alive	189	Alive
4043	Male	25	None	Standard Chemotherapy	Bu/Cy/ATG, 7+3 (Cytarabine, Idarubicin), HiDAC	Alive	444	Alive
4197	Female	45	Unknown	Standard Chemotherapy, Supportive/Palliative Care	7+3 (Cytarabine, Idarubicin), Sunitinib, FLAG-IDA (Cytarabine, Filgrastim, Fludarabine, Idarubicin), 7+3 (Cytarabine, Idarubicin) plus Sorafenib, Hydroxyurea	Dead	634	Dead-Disease
4201	Female	52	None	Standard Chemotherapy	MiDAC, FLAG-IDA plus Midostaurin, 7+3 (Cytarabine, Idarubicin) plus Dasatinib	Alive	414	Alive
4317	Female	72	Unknown	Targeted Therapy - Kinase Inhibitor(s)	Dasatinib	Unknown	Missing	Missing



Supplemental figure 1: Cases from the Beat AML sample cohort exome sequencing dataset where serial sampling was performed. Days on the x-axis. VAF = variant allele frequency, where 0 equals 0 and 1 equals 100% relative distribution. Blue dots indicate mutations in genes mutated in at least 10 samples, while grey dots indicate mutations in genes which were mutated in less than 10 samples.



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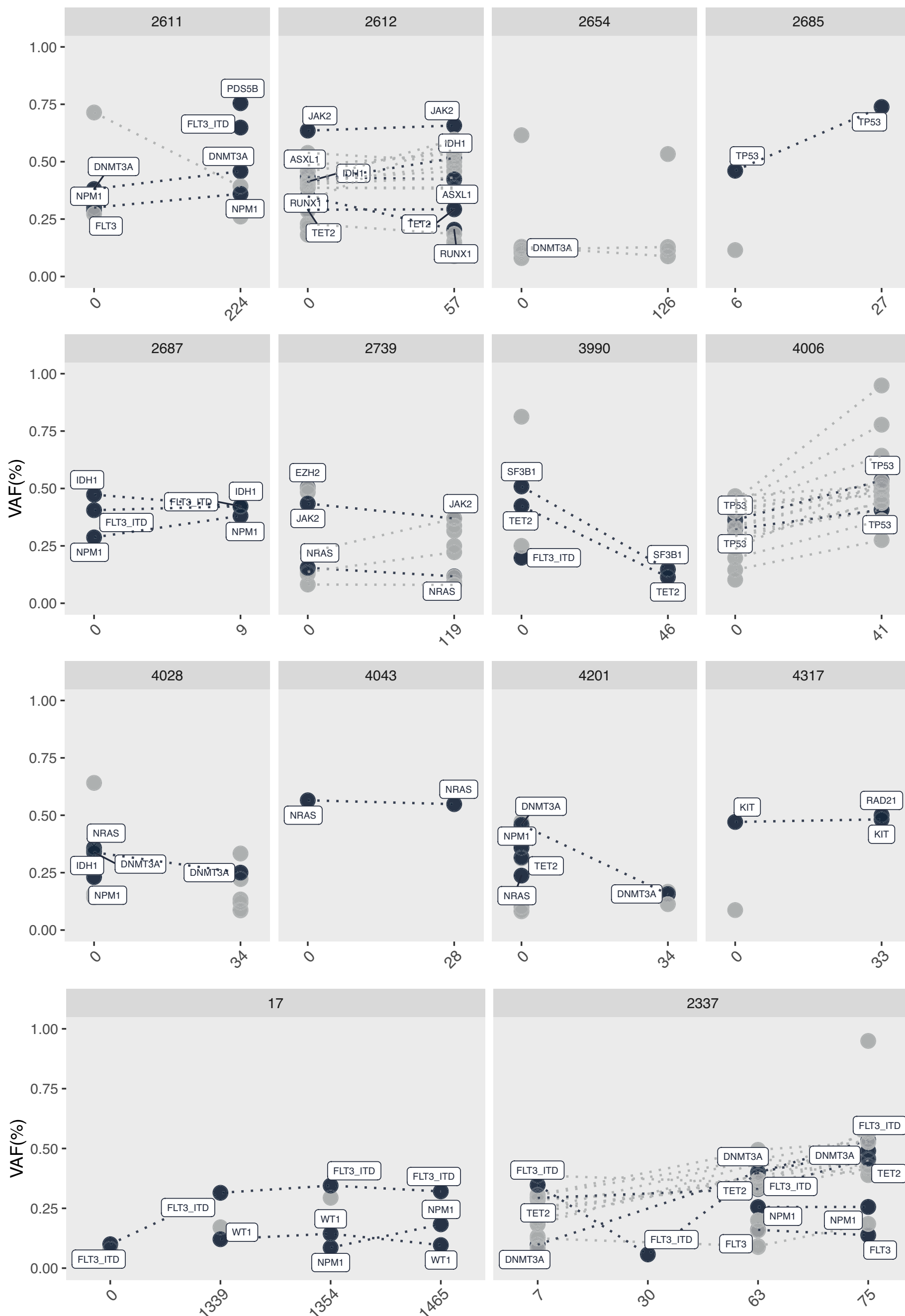

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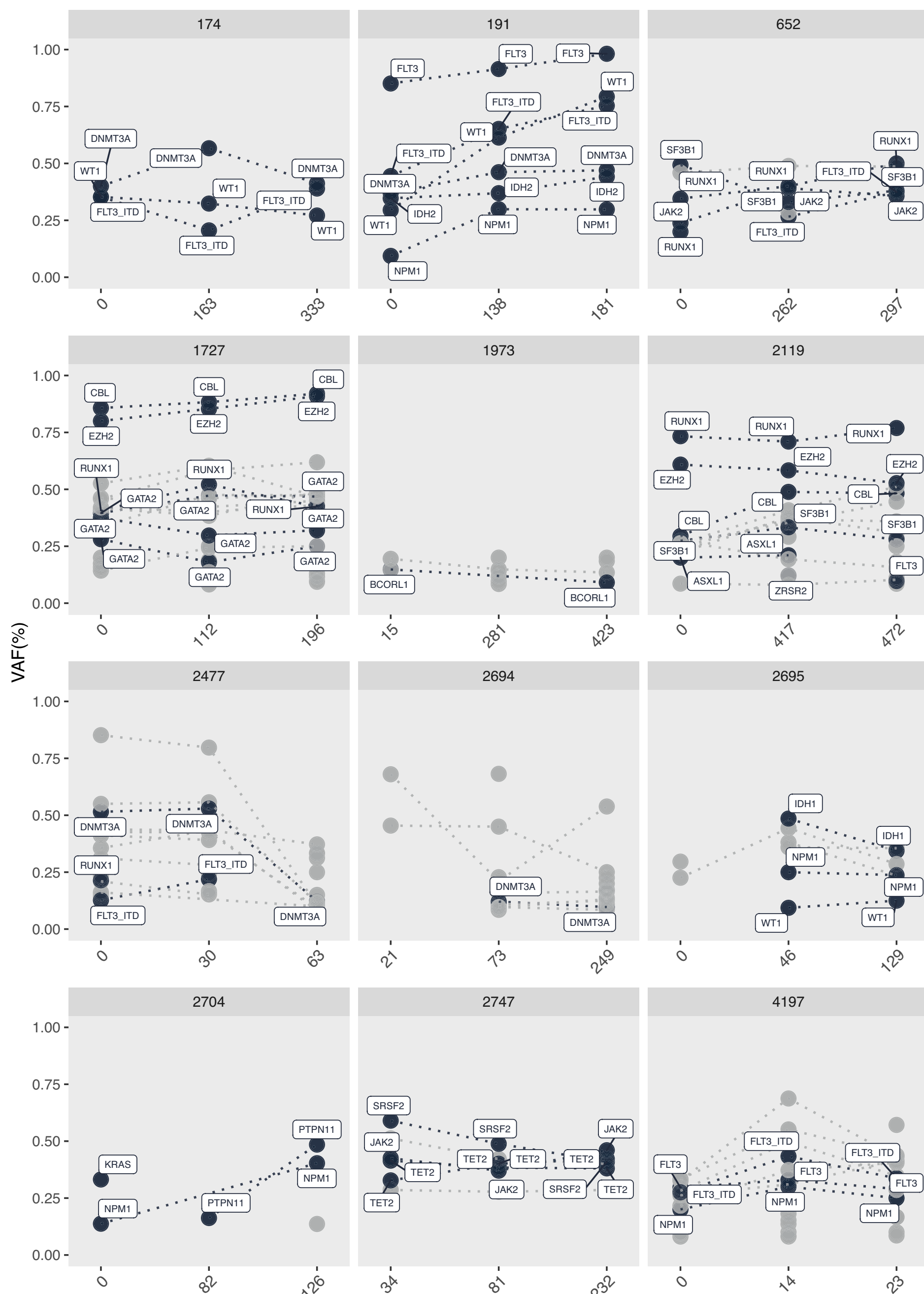
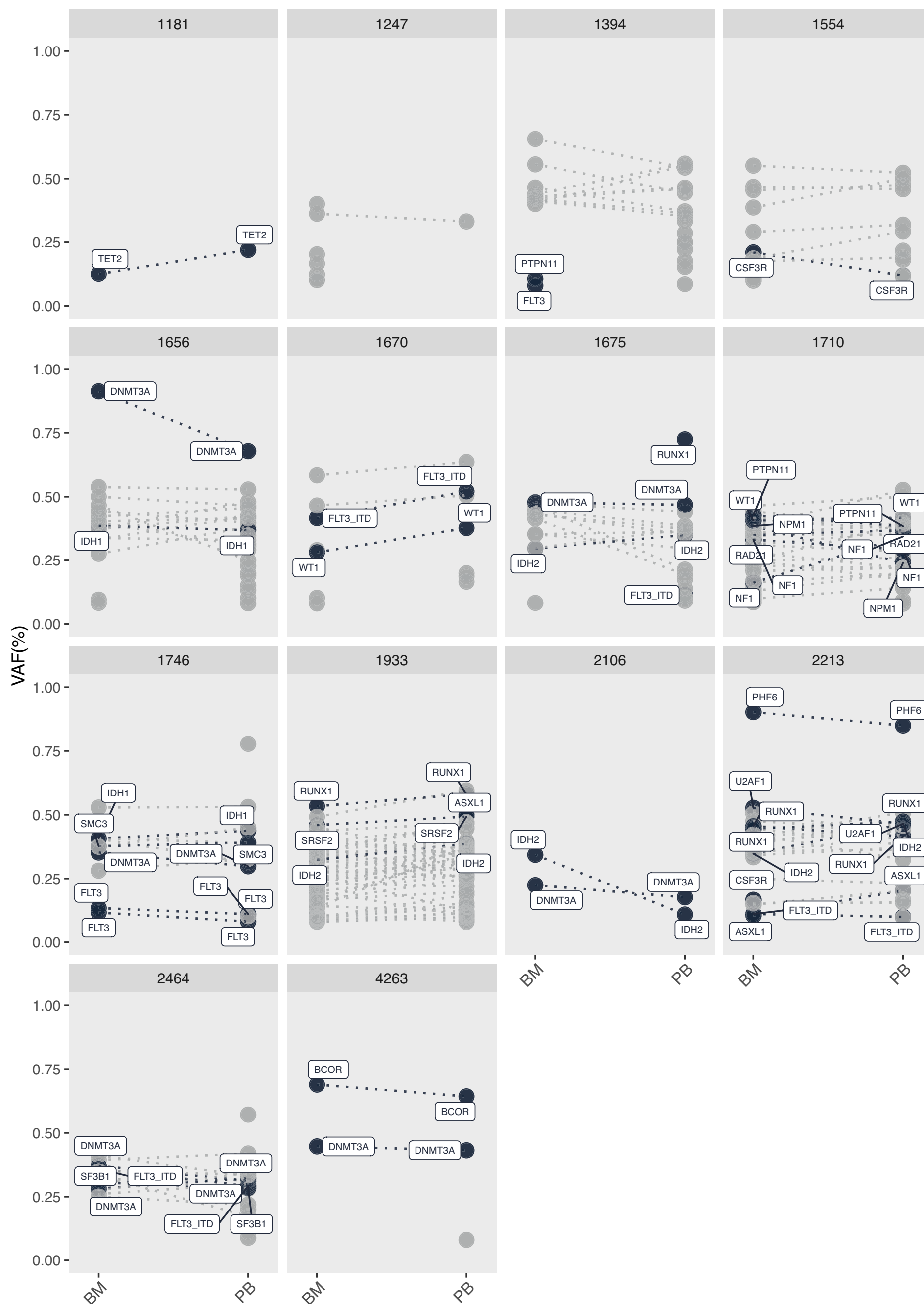


Supplemental figure 1: Cases from the Beat AML sample cohort exome sequencing dataset where serial sampling was performed. Days on the x-axis. VAF = variant allele frequency, where 0 equals 0 and 1 equals 100% relative distribution. Blue dots indicate mutations in genes mutated in at least 10 samples, while grey dots indicate mutations in genes which were mutated in less than 10 samples.

Supplementary figure 2 – Comparing bone marrow and peripheral blood samples in Beat-AML



Supplemental figure 2: 14 individual cases from the Beat AML sample cohort exome sequencing dataset where bone marrow (BM) and peripheral blood (PB) draws were acquired synchronously, allowing direct comparison. VAF = variant allele frequency, where 0 equals 0 and 1 equals 100% relative distribution. Blue dots indicate mutations in genes mutated in at least 10 samples, while grey dots indicate mutations in genes which were mutated in less than 10 samples.

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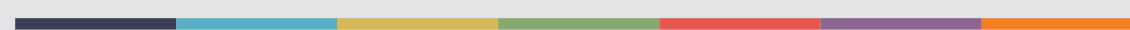

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