



Universiteit
Leiden
The Netherlands

Discovery of FLT3 inhibitors for the treatment of acute myeloid leukemia

Grimm, S.H.

Citation

Grimm, S. H. (2019, February 20). *Discovery of FLT3 inhibitors for the treatment of acute myeloid leukemia*. Retrieved from <https://hdl.handle.net/1887/68880>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/68880>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/68880> holds various files of this Leiden University dissertation.

Author: Grimm, S.H.

Title: Discovery of FLT3 inhibitors for the treatment of acute myeloid leukemia

Issue Date: 2019-02-20

Chapter 1

General introduction

On cancer

Cancer is used as an umbrella term for diseases in which cells in a multicellular organism are behaving differently than their tissue of origin due to a variety of root causes.¹⁻³ In these diseases this generally goes along with uncontrolled cell growth and cell proliferation, which in turn results in a diverse set of problems for the host organism, most prominently death. This makes cancer one of the leading causes of death throughout the world.^{1,4} While the development of many types of cancers is attributable to epidemiological factors, such as smoking,^{5,6} alcohol consumption,^{7,8} solar radiation^{9,10} and nutritional preferences,^{11,12} there are also general risk factors, namely age, partially through buildup of other risk factors.¹³ While the root causes vary, they all seem to cause mutations or genome rearrangements in different types of cells.¹⁴ Some of these mutations ultimately lead to the development of malignant cells, which are defined by six hallmarks: *sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis*.^{1,2,15} The occurrence of malignant cells seems to be governed by an evolutionary process, starting out with small genetic alterations, leading through acquired genetic instability and clone selection to the above described characteristics.¹⁶ This furthermore suggests that each cancer might be, at least on some level, unique. Most cancer cells constitute of numerous genetic abnormalities and the question which genetic alterations are responsible for malignancy is subject to some debate and is, depending on the type of cancer, not definitely answered.¹⁷ To differentiate between mutations responsible for malignancy and the ones that are not, the terms driver and passenger mutations have been defined.¹⁸ Driver mutations have a causal relation to oncogenesis, the formation of cancer and are frequently additionally responsible for cancer maintenance. To use the screening for, and identification of mutations as a starting point for

the development of targeted treatments, it is crucial to distinguish between driver and passenger mutations.¹⁸ Responsible for a vast cellular signaling network, mutations in protein kinases have frequently been named driver mutations in cancer progression.¹⁹

Protein kinases and kinase inhibitors

Post-translational modifications (PTMs) are covalent chemical modifications of proteins that occur during or after protein-synthesis. This process ensures the possibility for regulation of activity and function of cellular processes. There are a multitude of different enzyme classes described that facilitate PTMs and among them, protein kinases take up a prominent role.²⁰

Protein kinases are a closely related class of enzymes that are characterized by their common function to phosphorylate other proteins, most commonly on serine, threonine or tyrosine residues.^{21,22} The reverse process of this, the dephosphorylation is controlled by phosphatases. The transfer of the phosphate group causes structural changes in the target enzyme, directly influencing its activity or localization. If the target enzyme is also a kinase, a concerted signaling pathway may be created, which are often called kinase signaling cascades.²³ Through this system, cells can react or adapt to extracellular stimuli, involving many different cellular processes such as metabolic pathways or gene transcription. There are over 500 human kinases described in the human genome and many of them have been associated with driver mutations in cancer progression and as such qualify as drug targets.^{19,24} Not surprisingly, a sizeable portion of these kinases are involved in signaling pathways connected to cell proliferation and apoptosis.^{25–27}

This led to extensive efforts to develop compounds that can selectively inhibit kinase signaling in aberrant pathways. Currently, 38 kinase inhibitors (KIs) have been approved by the authorities.²⁶ The large number of kinases and the fact that most of KIs bind in the highly conserved adenosine triphosphate (ATP) binding pocket make the efforts to selectively inhibit one kinase in a complex cellular environment difficult. While there are more and less selective KIs, most clinically investigated KIs tend to inhibit at least several kinases.^{28,29} While profiling KIs in any complex environment, such as cells, off-target activity has always to be considered in the interpretation of the results.

Acute myeloid leukemia and FLT3

Acute myeloid leukemia (AML) is a group of hematopoietic disorders that are characterized by failure in differentiation and abnormal growth of hematopoietic stem cells, resulting in high levels of non-functional blood cells termed blast cells in the blood stream, leading to impaired blood flow, especially in capillaries, resulting in high mortality.^{30–33} Prognosis varies, but relapse rates remain high and especially older patients have a poor prognosis due to them not being able to cope with the standard chemotherapy.^{34,35} Untreated AML leads in almost all

cases to death within weeks to months and incident rates vary most prominently with age, steadily increasing to a peak at around 80 years. The median age of a newly diagnosed AML patient is 65 years old and the incident rate < 65 years olds is 1.8 and $\geq$ 65 years old is 17 per 100.000 persons (data from the U.S., 2000 - 2003).³⁶ Newly diagnosed AML is genetically diverse and several genetic aberrations are deemed clinically relevant, amongst them aberrations in nucleophosmin (NPM1) and CCAAT/enhancer-binding protein alpha (CEBPA) as well as fms-like tyrosine kinase 3 (FLT3).^{33,34}

FLT3, classified as a type 3 receptor tyrosine kinase together with PDGFR, c-KIT and FMS, consists of an extracellular domain of five immunoglobulin-like domains, a transmembrane domain, a cytosolic juxtamembrane domain and two intracellular kinase insert domain linked tyrosine kinase-domains and is normally expressed in myeloid and lymphoid progenitor cells.^{37–39} Upon extracellular activation by the FLT3 ligand (FL), FLT3 activates, like other members of the type 3 receptor tyrosine kinases, downstream signaling pathways via PI3K, AKT, STAT5, mTOR, RAS and ERK.^{39,40} 20 to 30% of AML patients harbor an internal tandem duplication mutation (ITD) in the juxtamembrane domain of FLT3 rendering the FLT3 activation and signaling FL binding-independent. The continued signaling of the mutated FLT3 receptor, possibly through alternate pathways than the wild-type counterpart, is thought to drive the proliferation of mutated cells growth-factor independent.⁴¹

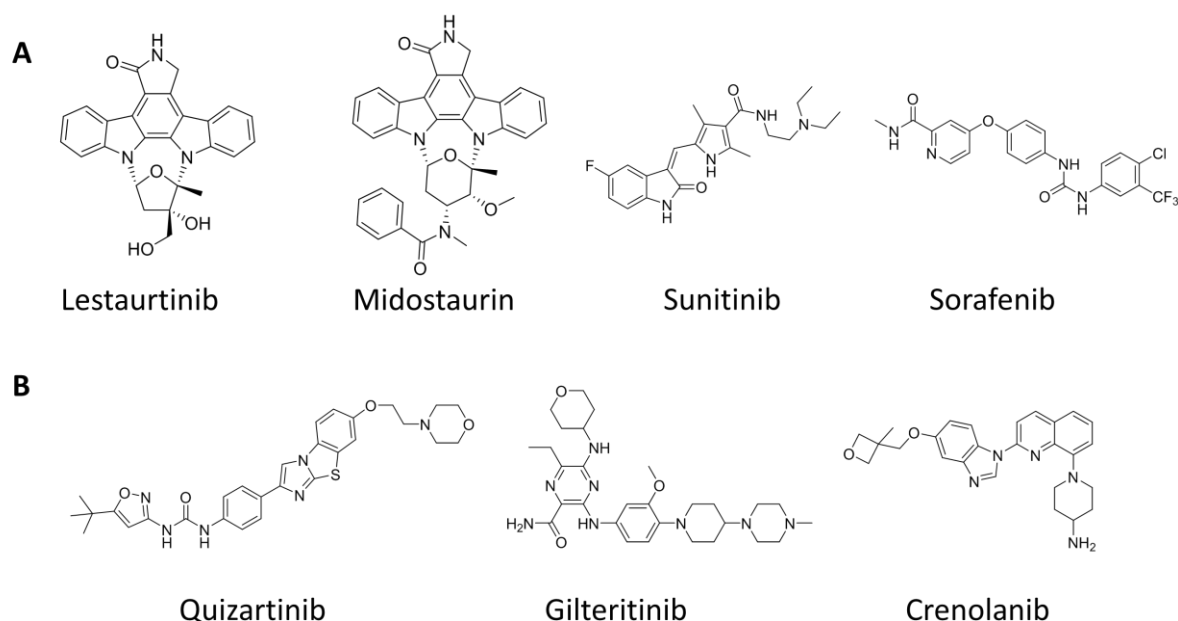


Figure 1: Clinically investigated first- (A) and second-generation (B) FLT3 inhibitors.

Numerous FLT3 inhibitors have been described and the most promising of them have been investigated in clinical trials.⁴² The to date clinically investigated inhibitors include the so called first generation FLT3 inhibitors, which were originally developed for other targets and repurposed as FLT3 inhibitors. First generation inhibitors are lestaurtinib, midostaurin, sunitinib and sorafenib (Figure 1A). Second generation inhibitors, such as quizartinib, crenolanib and gilteritinib, were specifically developed as FLT3 inhibitors (Figure 1B). While all

of these drugs are highly potent FLT3 inhibitors, their clinical efficacy varies substantially, which can be partially explained by different off-target and pharmacokinetic profiles.^{42–44} Different possible treatment regimens were investigated in clinical trials, including kinase inhibitor monotherapy or in combination with standard chemotherapy. Efficacy of the monotherapy trials varied from short lived partial response⁴⁵ to promising in the cases of quizartinib, crenolanib and gilteritinib.⁴³ The benefits of combination treatment, together with existing treatment regimens vary in a similar fashion from virtually non-existent with lestaurtinib⁴⁶ to sufficient to obtain market approval, as seen in midostaurin.^{47,48} A substantial number of clinical trials, are still ongoing and it remains to be seen if any drug candidates can show enough benefit for patients to warrant approval.

Complicating FLT3-ITD AML treatment is the emergence of drug resistance conferring mutations after FLT3 inhibitor treatment. This provides strong evidence that FLT3 mutations are driver mutations in AML progression, thereby validating FLT3 as a valuable target in AML treatment.^{31,49,50} Numerous mutations have been described, most notably in the amino acid residues of the activation loop (most frequently D835) and the gatekeeper residue (F691).^{49–52} These mutations cause structural changes, thereby decreasing inhibitor binding activity. To treat these emerging mutations, or to preemptively prevent their emergence at relapse, new chemical matter is needed that retains activity against the numerous possible mutant forms of FLT3, either as a single agent or as combination treatment.^{49,53,54}

Aim and outline of this thesis

The aim of this thesis is the discovery and optimization of novel FLT3 inhibitors that can be used to develop potential new treatments of drug-resistant acute myeloid leukemia (AML), as well as chemical biological techniques that facilitate their discovery.

Chapter 2 brings together modern techniques and describes the development of a chemical proteomics-based assay to determine kinase inhibitor off-target landscape in living cells.^{55,56} In this study five clinically investigated FLT3 inhibitors, namely sunitinib, quizartinib, crenolanib, gilteritinib and midostaurin, were tested for their off-target profile in two different AML cell lines: MV4-11 and U937. For numerous proteins cellular inhibitor-target engagement could be confirmed, and moreover new off-targets were discovered and subsequently validated. **Chapter 3** shows the comprehensive structure-activity relationship of a H-89-derived chemical series as FLT3 inhibitors for treatment of AML. Extensive structure-activity-relationships of the H-89 series provided insight into the binding mode, as well as the development of highly active, sub-nanomolar FLT3 inhibitors. **Chapter 4** describes the high throughput screening of more than 230,000 compounds to discover new chemical matter as FLT3 inhibitors that are less vulnerable to known clinically observed FLT3 mutations in the ATP-binding pocket. After the initial *in vitro* screen against FLT3, two deselection screens, dose response evaluation against FLT3 and an intensive literature study, 21 compounds were selected and tested in a total of seven human and murine cell lines to ensure activity against

mutant variations of the FLT3 gene. This culminated in the discovery of SPCE00476_01 and NP_004099_001. **Chapter 5** describes the optimization the novel hits discovered in Chapter 4. Design, synthesis and testing of a large array of compounds did not only lead to a good understanding of the structure-activity relationship of the chemical series, but also led to the discovery of cellular active, sub-nanomolar FLT3 inhibitors with appropriate physico-chemical properties, such as molecular weight and lipophilic efficiency. Two compounds were selected and further profiling of their off-target activity in AML cells using the chemical proteomics assay developed in Chapter 2, and pharmacokinetic properties in mice. **Chapter 6** summarizes this thesis and suggests possible future directions for AML research.

References

- (1) Greaves, M.; Maley, C. C. Clonal Evolution in Cancer. *Nature* **2012**, *481* (7381), 306–313.
- (2) Hanahan, D.; Weinberg, R. A. Hallmarks of Cancer: The next Generation. *Cell* **2011**, *144* (5), 646–674.
- (3) Siegel, R. L.; Miller, K. D.; Jemal, A. Cancer Statistics, 2018. *CA. Cancer J. Clin.* **2018**, *68* (1), 7–30.
- (4) Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D. M.; Forman, D.; Bray, F. Cancer Incidence and Mortality Worldwide: Sources, Methods and Major Patterns in GLOBOCAN 2012. *Int. J. Cancer* **2015**, *136* (5), E359–E386.
- (5) Sasco, A. J.; Secretan, M. B.; Straif, K. Tobacco Smoking and Cancer: A Brief Review of Recent Epidemiological Evidence. *Lung Cancer* **2004**, *45*, S3–S9.
- (6) Khuder, S. A. Effect of Cigarette Smoking on Major Histological Types of Lung Cancer: A Meta-Analysis. *Lung Cancer* **2001**, *31* (2–3), 139–148.
- (7) Nelson, D. E.; Jarman, D. W.; Rehm, J.; Greenfield, T. K.; Rey, G.; Kerr, W. C.; Miller, P.; Shield, K. D.; Ye, Y.; Naimi, T. S. Alcohol-Attributable Cancer Deaths and Years of Potential Life Lost in the United States. *Am. J. Public Health* **2013**, *103* (4), 641–648.
- (8) Schütze, M.; Boeing, H.; Pischon, T.; Rehm, J.; Kehoe, T.; Gmel, G.; Olsen, A.; Tjønneland, A. M.; Dahm, C. C.; Overvad, K.; et al. Alcohol Attributable Burden of Incidence of Cancer in Eight European Countries Based on Results from Prospective Cohort Study. *BMJ* **2011**, *342*, d1584.
- (9) de Gruijl, F. R. Skin Cancer and Solar UV Radiation. *Eur. J. Cancer* **1999**, *35* (14), 2003–2009.
- (10) Young, C. Solar Ultraviolet Radiation and Skin Cancer. *Occup. Med. (Chic. Ill.)*. **2009**, *59* (2), 82–88.
- (11) Ackerman, S. E.; Blackburn, O. A.; Marchildon, F.; Cohen, P. Insights into the Link Between Obesity and Cancer. *Curr. Obes. Rep.* **2017**, *6* (2), 195–203.
- (12) Arnold, M.; Leitzmann, M.; Freisling, H.; Bray, F.; Romieu, I.; Renehan, A.; Soerjomataram, I. Obesity and Cancer: An Update of the Global Impact. *Cancer Epidemiol.* **2016**, *41*, 8–15.
- (13) Whiteman, D. C.; Wilson, L. F. The Fractions of Cancer Attributable to Modifiable Factors: A Global Review. *Cancer Epidemiol.* **2016**, *44*, 203–221.
- (14) Loeb, L. A.; Harris, C. C. Advances in Chemical Carcinogenesis: A Historical Review and Prospective. *Cancer Res.* **2008**, *68* (17), 6863–6872.
- (15) Hanahan, D.; Weinberg, R. A. The Hallmarks of Cancer. *Cell* **2000**, *100* (1), 57–70.
- (16) Nowell, P. C. The Clonal Evolution of Tumor Cell Populations. *Science* **1976**, *194* (4260), 23–28.
- (17) Stratton, M. R. Exploring the Genomes of Cancer Cells: Progress and Promise. *Science*

- (80-). **2011**, 331 (6024), 1553–1558.
- (18) Stratton, M. R.; Campbell, P. J.; Futreal, P. A. The Cancer Genome. *Nature* **2009**, 458 (7239), 719–724.
- (19) Cohen, P. Protein Kinases — the Major Drug Targets of the Twenty-First Century? *Nat. Rev. Drug Discov.* **2002**, 1 (4), 309–315.
- (20) Walsh, C. T.; Garneau-Tsodikova, S.; Gatto, G. J. Protein Posttranslational Modifications: The Chemistry of Proteome Diversifications. *Angew. Chemie Int. Ed.* **2005**, 44 (45), 7342–7372.
- (21) And, L. N. J.; Lewis, R. J. Structural Basis for Control by Phosphorylation. *Chem. Rev.* **2001**, 101 (8), 2209–2242.
- (22) Schwartz, P. A.; Murray, B. W. Protein Kinase Biochemistry and Drug Discovery. *Bioorg. Chem.* **2011**, 39 (5–6), 192–210.
- (23) Fabbro, D.; Cowan-Jacob, S. W.; Moebitz, H. Ten Things You Should Know about Protein Kinases: IUPHAR Review 14. *Br. J. Pharmacol.* **2015**, 172 (11), 2675–2700.
- (24) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The Protein Kinase Complement of the Human Genome. *Science* **2002**, 298 (5600), 1912–1934.
- (25) Cohen, P.; Alessi, D. R. Kinase Drug Discovery – What’s Next in the Field? *ACS Chem. Biol.* **2013**, 8 (1), 96–104.
- (26) Wu, P.; Nielsen, T. E.; Clausen, M. H. FDA-Approved Small-Molecule Kinase Inhibitors. *Trends Pharmacol. Sci.* **2015**, 36 (7), 422–439.
- (27) Brognard, J.; Hunter, T. Protein Kinase Signaling Networks in Cancer. *Curr. Opin. Genet. Dev.* **2011**, 21 (1), 4–11.
- (28) Klaeger, S.; Heinzlmeir, S.; Wilhelm, M.; Polzer, H.; Vick, B.; Koenig, P. A.; Reinecke, M.; Ruprecht, B.; Petzoldt, S.; Meng, C.; et al. The Target Landscape of Clinical Kinase Drugs. *Science (80-)*. **2017**, 358 (6367), eaan4368.
- (29) Müller, S.; Chaikuad, A.; Gray, N. S.; Knapp, S. The Ins and Outs of Selective Kinase Inhibitor Development. *Nat. Publ. Gr.* **2015**, 11 (11), 818–821.
- (30) Thiede, C.; Steudel, C.; Mohr, B.; Schaich, M.; Schäkel, U.; Platzbecker, U.; Wermke, M.; Bornhäuser, M.; Ritter, M.; Neubauer, A.; et al. Analysis of FLT3-Activating Mutations in 979 Patients with Acute Myelogenous Leukemia: Association with FAB Subtypes and Identification of Subgroups with Poor Prognosis. *Blood* **2002**, 99 (12), 4326–4335.
- (31) König, H.; Levis, M. Targeting FLT3 to Treat Leukemia. *Expert Opin. Ther. Targets* **2015**, 19 (1), 37–54.
- (32) Shipley, J. L.; Butera, J. N. Acute Myelogenous Leukemia. *Exp. Hematol.* **2009**, 37 (6), 649–658.
- (33) Döhner, H.; Weisdorf, D. J.; Bloomfield, C. D. Acute Myeloid Leukemia. *N. Engl. J. Med.* **2015**, 373 (12), 1136–1152.
- (34) Döhner, H.; Estey, E. H.; Amadori, S.; Appelbaum, F. R.; Büchner, T.; Burnett, A. K.;

- Dombret, H.; Fenaux, P.; Grimwade, D.; Larson, R. A.; et al. Diagnosis and Management of Acute Myeloid Leukemia in Adults: Recommendations from an International Expert Panel, on Behalf of the European LeukemiaNet. *Blood* **2010**, *115* (3), 453–474.
- (35) Jongen-Lavrencic, M.; Grob, T.; Hanekamp, D.; Kavelaars, F. G.; al Hinai, A.; Zeilemaker, A.; Erpelinck-Verschueren, C. A. J.; Gradowska, P. L.; Meijer, R.; Cloos, J.; et al. Molecular Minimal Residual Disease in Acute Myeloid Leukemia. *N. Engl. J. Med.* **2018**, *378* (13), 1189–1199.
- (36) Deschler, B.; Lübbert, M. Acute Myeloid Leukemia: Epidemiology and Etiology. *Cancer* **2006**, *107* (9), 2099–2107.
- (37) Griffith, J.; Black, J.; Faerman, C.; Swenson, L.; Wynn, M.; Lu, F.; Lippke, J.; Saxena, K. The Structural Basis for Autoinhibition of FLT3 by the Juxtamembrane Domain. *Mol. Cell* **2004**, *13* (2), 169–178.
- (38) Scheijen, B.; Griffin, J. D. Tyrosine Kinase Oncogenes in Normal Hematopoiesis and Hematological Disease. *Oncogene* **2002**, *21* (21), 3314–3333.
- (39) Stirewalt, D. L.; Radich, J. P. The Role of FLT3 in Haematopoietic Malignancies. *Nat. Rev. Cancer* **2003**, *3* (9), 650–665.
- (40) El Fakih, R.; Rasheed, W.; Hawsawi, Y.; Alsermani, M.; Hassanein, M.; El Fakih, R.; Rasheed, W.; Hawsawi, Y.; Alsermani, M.; Hassanein, M. Targeting FLT3 Mutations in Acute Myeloid Leukemia. *Cells* **2018**, *7* (1), 4.
- (41) Lange, B.; Valtieri, M.; Santoli, D.; Caracciolo, D.; Mavilio, F.; Gemperlein, I.; Griffin, C.; Emanuel, B.; Finan, J.; Nowell, P. Growth Factor Requirements of Childhood Acute Leukemia: Establishment of GM-CSF-Dependent Cell Lines. *Blood* **1987**, *70* (1), 192–199.
- (42) Garcia, J. S.; Stone, R. M. The Development of FLT3 Inhibitors in Acute Myeloid Leukemia. *Hematol. Oncol. Clin. North Am.* **2017**, *31* (4), 663–680.
- (43) Leick, M. B.; Levis, M. J. The Future of Targeting FLT3 Activation in AML. *Curr. Hematol. Malig. Rep.* **2017**, *12* (3), 153–167.
- (44) König, H.; Levis, M. Targeting FLT3 to Treat Leukemia. **2015**, 37–54.
- (45) Fiedler, W.; Serve, H.; Döhner, H.; Schwittay, M.; Ottmann, O. G.; O’Farrell, A.-M.; Bello, C. L.; Allred, R.; Manning, W. C.; Cherrington, J. M.; et al. A Phase 1 Study of SU11248 in the Treatment of Patients with Refractory or Resistant Acute Myeloid Leukemia (AML) or Not Amenable to Conventional Therapy for the Disease. *Blood* **2005**, *105* (3), 986–993.
- (46) Levis, M.; Ravandi, F.; Wang, E. S.; Baer, M. R.; Perl, A.; Coutre, S.; Erba, H.; Stuart, R. K.; Baccarani, M.; Cripe, L. D.; et al. Results from a Randomized Trial of Salvage Chemotherapy Followed by Lestaurtinib for Patients with FLT3 Mutant AML in First Relapse. *Blood* **2011**, *117* (12), 3294–3301.
- (47) Kayser, S.; Levis, M. J. Advances in Targeted Therapy for Acute Myeloid Leukaemia. *Br. J. Haematol.* **2018**, *180* (4), 484–500.
- (48) Levis, M. Midostaurin Approved for FLT3-Mutated AML. *Blood* **2017**, *129* (26), 3403–3406.

-
- (49) Smith, C. C.; Wang, Q.; Chin, C.-S.; Salerno, S.; Damon, L. E.; Levis, M. J.; Perl, A. E.; Travers, K. J.; Wang, S.; Hunt, J. P.; et al. Validation of ITD Mutations in FLT3 as a Therapeutic Target in Human Acute Myeloid Leukaemia. *Nature* **2012**, *485* (7397), 260–263.
- (50) Smith, C. C.; Zhang, C.; Lin, K.; Lasater, E. A.; Zhang, Y.; Massi, E.; Damon, E.; Pendleton, M.; Bashir, A.; Sebra, R.; et al. Characterizing and Overriding the Structural Mechanism of the Quizartinib-Resistant FLT3 “Gatekeeper” F691L Mutation with PLX3397. *Cancer Discov.* **2015**, *5* (6), 668–679.
- (51) Man, C. H.; Fung, T. K.; Ho, C.; Han, H. H. C.; Chow, H. C. H.; Ma, A. C. H.; Choi, W. W. L.; Lok, S.; Cheung, A. M. S.; Eaves, C.; et al. Sorafenib Treatment of FLT3-ITD(+) Acute Myeloid Leukemia: Favorable Initial Outcome and Mechanisms of Subsequent Nonresponsiveness Associated with the Emergence of a D835 Mutation. *Blood* **2012**, *119* (22), 5133–5143.
- (52) Yamamoto, Y.; Kiyoi, H.; Nakano, Y.; Suzuki, R.; Kidera, Y.; Miyawaki, S.; Asou, N.; Kuriyama, K.; Yagasaki, F.; Shimazaki, C.; et al. Activating Mutation of D835 within the Activation Loop of FLT3 in Human Hematologic Malignancies. *Blood* **2001**, *97* (8), 2434–2439.
- (53) Man, C. H.; Fung, T. K.; Ho, C.; Han, H. H. C.; Chow, H. C. H.; Ma, A. C. H.; Choi, W. W. L.; Lok, S.; Cheung, A. M. S.; Eaves, C.; et al. Sorafenib Treatment of FLT3-ITD+ Acute Myeloid Leukemia: Favorable Initial Outcome and Mechanisms of Subsequent Nonresponsiveness Associated with the Emergence of a D835 Mutation. *Blood* **2012**, *119* (22), 5133–5143.
- (54) Smith, C. C.; Lasater, E. a.; Zhu, X.; Lin, K. C.; Stewart, W. K.; Damon, L. E.; Salerno, S.; Shah, N. P. Activity of Ponatinib against Clinically-Relevant AC220-Resistant Kinase Domain Mutants of FLT3-ITD. *Blood* **2013**, *121* (16), 3165–3171.
- (55) Zhao, Q.; Ouyang, X.; Wan, X.; Gajiwala, K. S.; Kath, J. C.; Jones, L. H.; Burlingame, A. L.; Taunton, J. Broad-Spectrum Kinase Profiling in Live Cells with Lysine-Targeted Sulfonyl Fluoride Probes. *J. Am. Chem. Soc.* **2017**, *139* (2), 680–685.
- (56) van Rooden, E. J.; Florea, B. I.; Deng, H.; Baggelaar, M. P.; van Esbroeck, A. C. M.; Zhou, J.; Overkleeft, H. S.; van der Stelt, M. Mapping in Vivo Target Interaction Profiles of Covalent Inhibitors Using Chemical Proteomics with Label-Free Quantification. *Nat. Protoc.* **2018**, *13* (4), 752–767.