

Developmental Awards Candidate Leukemia Spore

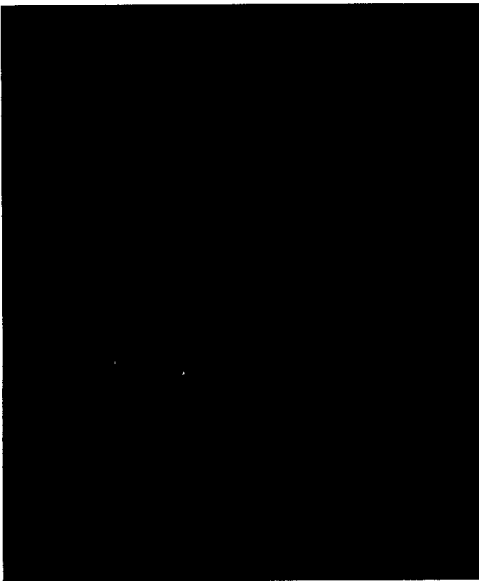
**William G. Bornmann,**  
*Professor*

**Department of Experimental Diagnostic  
Imaging**

## **Designing Inhibitors to c-Kit, Flt3, PDGFRa/PDGFRb and c-Met by Structure-Based Drug Design for the Therapeutic Imaging of Acute Myeloid Leukemia.**

**Background:** "Acute myeloid leukemia (AML) remains the most common form of leukemia and the most common cause of leukemia death. While conventional chemotherapy can cure 25-45% of AML patients, most patients will either die of relapse or die from complications associated with treatment." This statement appeared in Blood Reviews (2003) and is a stunning reminder of the need to develop new specific and less toxic treatments. In order to achieve the goal, a basic understanding of the molecular biology of neoplastic transformation in which multiple genetic defects such as translocation, mutations within oncogenes, growth factor receptors have been implicated in the development of AML. Thus it is safe to say that many of these genetic defects have been identified as key components of signaling pathways responsible for proliferation and differentiation. Of these key components, protein tyrosine kinases have been identified to play instrumental roles in downstream signal transduction pathways, which influence cellular processes that maintain the delicate balance between proliferation, differentiation, senescence and apoptosis thus providing potential targets for intervention. This has been demonstrated in the treatment of CML with Imatinib (STI-571). Recently, several publications have clearly demonstrated the importance in targeting select tyrosine kinases. The first being Tefferi et al who has demonstrated the inhibition of PDGF and c-Kit by Imatinib (STI-571) and its clinical relevance in myeloid disorders such as acute myeloid leukemia, myeloid metaplasia, polycythemia vera and myelofibrosis. A second by Stone et al who demonstrated the clinical relevance of the FLT3 inhibitor PKC412 in acute myeloid leukemia. The third by Weimar et al demonstrates the involvement of c-Met in acute myeloid leukemia.

**Preliminary Data:** As part of an ongoing program to develop new chemotherapeutic agents based on tyrosine kinase inhibitors, the pyrido[2,3-*d*]pyrimidine class of kinase inhibitors which was initially developed as inhibitors of PDGFR, FGFR, EGFR and c-Src inhibitors attracted our interest having observed that the pyridopyrimidines were nM level IC<sub>50</sub> Abl kinase inhibitors. This strongly suggested that this class of inhibitor might have potential as a chemotherapeutic agent for the Bcr-Abl dependent cancer, chronic myelogenous leukemia, or CML. A number of these pyridopyrimidine have now been examined *in vitro* using purified Bcr-Abl and Bcr-Abl expressing cell lines, MO7ep210 and K-562. Also, BT-474, PC-3 and A-172 cell growth was inhibited at IC<sub>50</sub>'s of mid nM to low  $\mu$ M. Thus we were able to develop the pyrido[2,3-*d*]pyrimidines class of compounds as new potent inhibitors of Bcr-Abl and c-Kit tyrosine kinases, with significant inhibition (Table 1). In 2002, we (Bornmann and Kuriyan) published<sup>4</sup> the first X-ray co-crystal structures of PD173955 bound to Abl kinase domain (AblK:PD173955) in the active form (Figure 1)<sup>5</sup>. In addition we have found activity in other cell lines<sup>6</sup>.



**Comparison of the IC50's of tyrosine kinase inhibitors**

		STI571 (Gleevec)	PD173955	PD166326	CPD10 SU4314	CPD13
BCRABL	In Vitro	50 nM	1-2 nM	0.5 nM	25-50 μM	25 μM
	In Vivo	50 nM	1-2 nM	0.25 nM	25-50 μM	~20 μM
C-KIT	In Vitro	100 nM	50 nM	25 nM	~250 nM	5 μM
	In Vivo	100 nM	50 nM	25 nM	250 nM	0.5 nM
PDGFR	In Vitro	300 nM	1.6 μM	140 nM	5 μM	10 μM
	In Vivo	350 nM	300 nM	200 nM	ND	ND
EGFR	In Vitro	>10 μM	ND	82 nM	ND	ND
	In Vivo	ND	400 nM	400 nM	ND	ND

Table 1

Figure

Molecular modeling of PD173955 into the crystal structure for the c-Kit kinase domain by McRee et al demonstrates the unique fit of PD173955 into the ATP binding site (Figure 2)

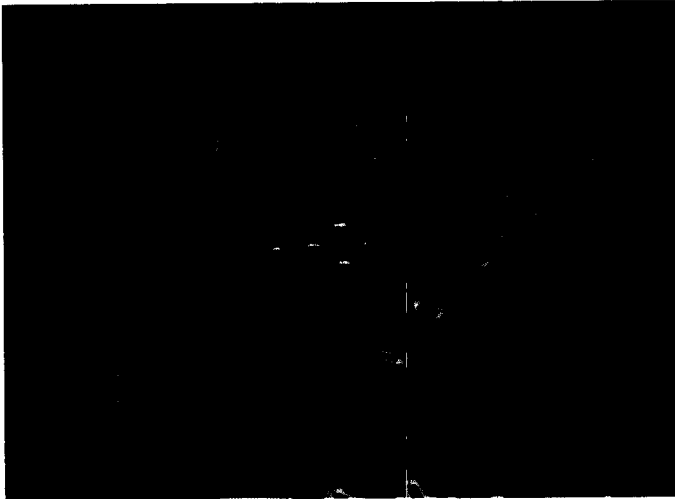


Figure 2.

While the pyrido[2,3-*d*]pyrimidine class of kinase inhibitors demonstrate a unique inhibitory effect on c-Kit. There are several limitations to their use; non-withstanding is their insoluble nature. Thus the focus of this proposal will be to develop new and novel pharmacokinetically optimized small molecule inhibitors for c-Kit, Flt3, PDGFR $\alpha$ /PDGFR $\beta$  and c-Met based on the above clinical observations.

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***Because of space considerations, the rest of this proposal concentrates on c-Kit as a representative of a proof of concept for the development of inhibitors of Flt3, PDGFR $\alpha$ /PDGFR $\beta$  and c-Met***

The current lack of diagnostic assays and markers predictive of sensitivity to c-KIT inhibitors limits the possibility of proper selection of patients for clinical trials aimed at the assessment of drugs targeting the c-Kit kinase. Thus, serious clinical challenges exist for the selection of optimal setting(s) in which to test and monitor both the biological and therapeutic efficacy of these novel drugs including: a) the identification of patients which would most favorably respond to therapy with c-Kit kinase inhibitors; b) individualization of therapeutic dose(s) and therapeutic regimens; and c) proper integration of these novel drugs into established cytotoxic therapeutic protocols; d) effective markers and methods for non-invasive monitoring of early efficacy of therapy.

Non-invasive PET imaging with c-Kit/PDGF kinase-specific radiolabeled agents could provide a better assessment of the levels and heterogeneity of c-Kit expression and activity in tumors in individual patients and provide selection criteria for inclusion of patients into re-designed clinical trials. Also, the ability to monitor the level of expression and activity of c-Kit at kinase level should provide a direct measure of biological drug efficacy (kinase inhibition) in tumors as opposed to surrogate tissues (e.g., hair, skin, etc.) before any therapeutic effect is to be expected. With this in mind we propose to develop a number of novel radiolabeled c-Kit -kinase-specific agents for SPET and PET imaging. These agents are will be based on new scaffolds and not on the pyrido[2,3-*d*]pyrimidines which will bind the c-Kit kinase ATP-binding pocket.

We also hypothesize that PET imaging with [ $^{124}$ I]c-Kit -kinase-specific inhibitor

could help to identify acute myeloid leukemia patients with high c-Kit expression/activity, as having higher [ $^{124}\text{I}$ ]c-Kit -kinase-specific inhibitor uptake and retention levels, and who would respond favorably to therapy with c-Kit c-Kit -kinase-specific abased inhibitors - both in terms of an early decrease in [ $^{124}\text{I}$ ] inhibitor uptake and retention, as well as by a gradual regression in tumor size (if the c-Kit signaling represents a dominant maintenance pathway). Another hypothesis behind this project is that PET imaging with [ $^{124}\text{I}$ ]c-Kit -kinase-specific inhibitor may help to identify acute myeloid leukemia patients with low c-Kit expression/activity, as those having a low [ $^{124}\text{I}$ ] inhibitor uptake and retention, and would respond poorly to therapy with c-Kit specific inhibitors.

In order to achieve the development of an effective inhibitor of c-Kit we will undertake the construction of libraries that will be guided by structure-based drug design based on the newly available crystal structure. Thus the proposal will have three specific aims to ensure a high likelihood of success.

**Aim 1.** Structure-based drug design by molecular modeling in which designs will be based on (1) existing synthetic cores known to work in other kinases and (2) novel cores discovered during initial screening of commercially available compounds. The first method of design based on existing cores is straightforward. Initially, a diverse set of R groups will be utilized to build a combinatorial library that will be screened *in-silico* by docking the enumerated combinations and scoring them against each kinase. Structures showing the highest scores by the consensus of multiple scoring methods will be synthesized and screened experimentally. Results from the screens will feed into the design of future compounds, which will gradually progress from a diverse set of compounds to a more focused set. The second method involves the screening of a large diverse library to determine “novel” compounds and synthetic cores.

**Aim 2.** The synthesis of focused libraries of novel pharmacokinetically optimized small molecule inhibitors for c-Kit by parallel solid phase or parallel solution phase chemistry.

**Aim 3.** We will perform high throughput screening to identify lead candidates as potent highly selective inhibitors of c-Kit and re-screen the lead candidates with high content screening against a panel of other receptor and cytoplasmic tyrosine kinases. This process will be designed to take into consideration the known mutations in the ATP binding site of c-Kit and selectivity in the targeting of c-Kit either alone or as part of a selected group whose members will be Flt3, PDGFR $\alpha$ /PDGFR $\beta$  and c-Met as well.

## **Plan of Research**

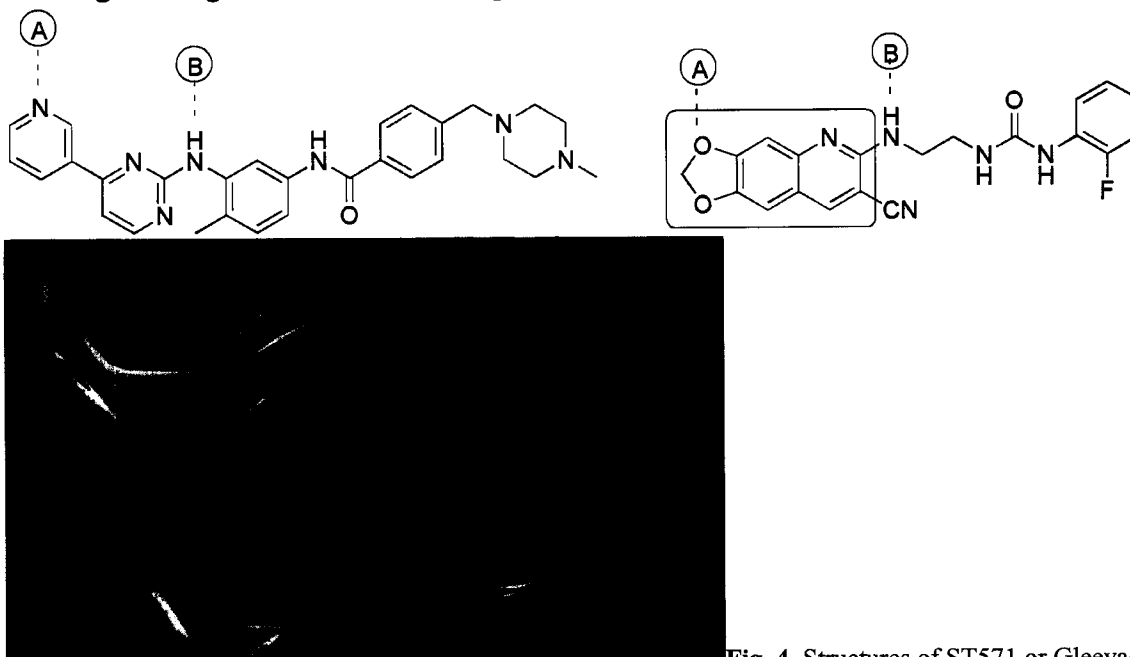
### **Aim 1. Structure-Based Drug Design of a Novel c-Kit Inhibitor by Molecular Modeling**

**Screening kinase specific libraries.** Several chemical database vendors offer kinase specific libraries that are presumably enriched with compounds that will give higher hit ratios when purchased and tested in assays designed to test for kinase activity. All compounds from these libraries will be screened *in-silico*. The resultant scores from

docking will be used to bias the selection of compounds to order. Screening of these compounds against assays for c-Kit, Flt3, PDGFR $\alpha$ /PDGFR $\beta$  and c-Met will provide initial data to develop SAR models and push ligand design. Additionally, analysis of the top scoring compounds will lead to a consensus of synthetic cores that will be used as the foundation for combinatorial efforts. Some efforts have already been initiated towards

**Fig. 3.** Ribbon/Tube diagram of c-Kit binding site with high scoring ligand, AST-6536377, drawn in ball & stick

this goal with the screening of a kinase specific library from Asinex. The 2D structures in the Kinase Targeted Library dated May 19, 2003 was screened against c-Kit kinase by FlexX. The top 10% compounds by consensus scoring were viewed in the docking site along with the crystallized ligand, STI-571. Shown in Fig. 3 is AST-6536377, one of top scoring compounds found from this library, docked into the binding site of c-Kit. Upon funding of this grant, arrangements will be made to acquire a portion of these commercially available compounds and screen other kinase specific libraries being made available. At the same time, internal efforts on focused libraries will begin. Several structural cores were observed to align well with the pyridine and pyrimidine rings of STI-571 and provide a similar H-bonding pattern in this region of the binding site. A 2D diagram showing this alignment is shown in Fig. 4.



**Fig. 4.** Structures of ST571 or Gleevac (left) and high scoring compound AST-6536377 (right). The dashed lines indicate hydrogen bonds.

Several of these structural cores are being actively investigated for feasibility in building a focused library, and the synthetic details of these libraries are discussed in the synthesis section.

**Screening general libraries.** We have constructed structural databases containing > 6 million that consist of available compounds from over 30 commercial vendors of chemical databases. A large proportion of these molecules are specifically designed for

early stage screening and therefore we expect to obtain *in-silico* hits from these databases, albeit at a reduced rate in comparison to the kinase specific libraries. However, structural diversity will offset the reduced hit rate and lead to the discovery of more novel synthetic cores, which will ultimately translate into more novel compounds. Due to enormous size of the screening library, some degree of pre-filtering will be necessary. The pre-filtering is generally many orders of a magnitude faster than the docking, which can take up to a few seconds/compound. This pre-filter will consist of a pharmacophore feature model built specifically for each kinase. The exact implementation has not been determined, but initial work has suggested a reduction to ~1-10% of the entire database.

**De novo Ligand Design.** The most novel compounds for this project will result from designs based on in-house synthetic expertise. We have a close collaboration between the synthetic chemistry and modeling that will allow for the creation of virtual libraries based on cores familiar to or easily accessible to the synthetic chemists. This will ensure a quick turnaround from a virtual hit to a real compound that can be tested *in-vitro* in the kinase assays.

**Kinase Selectivity.** There are well over 500 putative protein kinases and crystal structures of the catalytic domain are known for approximately 150 of them. The sheer number of possible kinases and similarity in their ATP binding sites (the principle target of most inhibitor design projects) leads to an important question of how to design for selectivity between them. An example that clearly demonstrates this challenge is insulin-like growth factor 1 (IGFR1RK), where selectivity is needed over insulin receptor kinase (IRK), yet the sequence identity is nearly 100% in the ATP binding cleft. In some cases, such as for Gleevac, inhibiting more than one kinase has a benefit to treating multiple diseases, but this was more fortuitous rather than by design. From this, it would seem clear that designing for kinase selectivity will be important. We aim to tackle this in a similar manner that other research groups have done, by optimizing against the selectivity region.

**X-ray Crystal and Homology Model Structures.** X-ray crystal structures of the kinase domain for c-Kit and c-Met are available from the PDB. Models for the other kinase structures will be constructed *via* Homology Modeling, using known kinase crystal structures as templates.

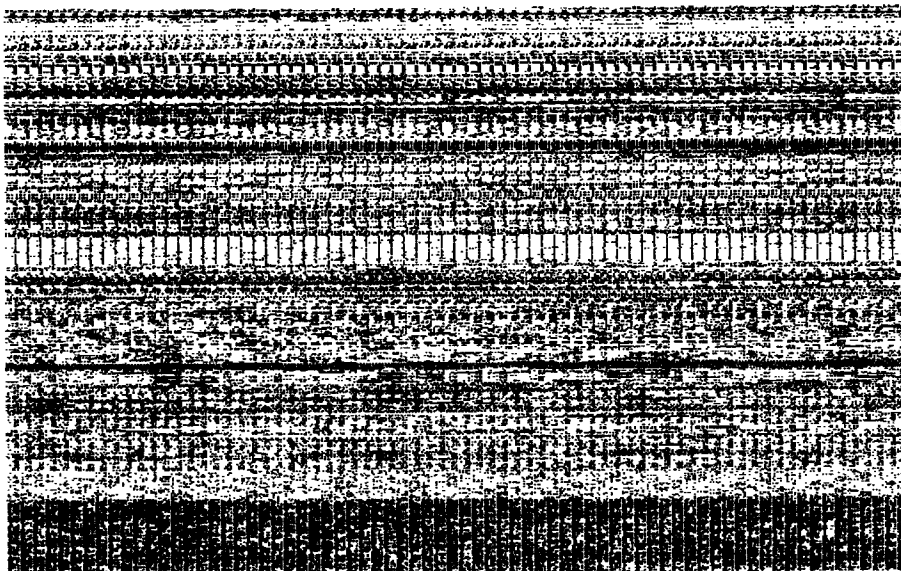
All modeling calculations were conducted on a four-processor MIPS R16000 Silicon Graphics Tezro running Sybyl. In particular, the Base and Biopolymer modules were used to prepare protein structures, and FlexX was used to dock ligands into those structures. Unless otherwise stated, docking setup consisted of reading in the structure and defining an active site consisting of residues within 5 Å of the ligand (when present) or residues known to interact with the various regions in the ATP binding site. All available methods were utilized in scoring the docked ligands and CSCORE, a consensus scoring method, was utilized to select the top candidates. For the creation of the structural databases, the Unity and Concord modules were used. Typically, compounds libraries

were received in the SDF file format and converted to a 3D Unity database, which was then exported to a format compatible with the docking program FlexX.

*Note that in the course of this work, all known point mutations of c-Kit will be modeled and docked with the candidate inhibitors in an effort to address the aspect of resistance. In addition, the c-Kit kinase inhibitor candidates will be further docked into the Flt3, PDGFR $\alpha$ /PDGFR $\beta$  and c-Met crystal structures/homology models in an effort to design inhibitors which can be either a selective inhibitor of c-Kit only, or an inhibitor which can bind selectively to any combination of Flt3, PDGFR $\alpha$ /PDGFR $\beta$  and c-Met or all.*

**Aim 2. The synthesis of focused libraries of novel pharmacokinetically optimized small molecule inhibitors for c-Kit by parallel solid phase or parallel solution phase chemistry.**

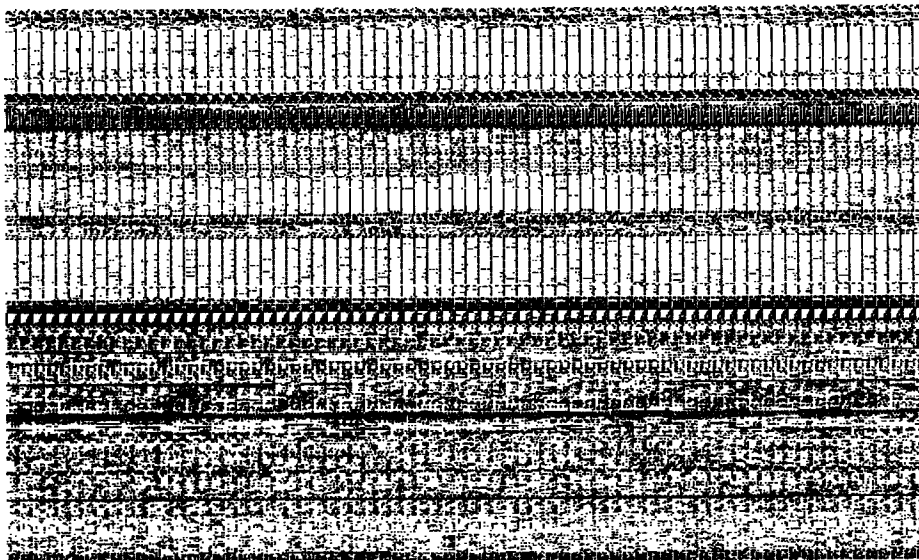
In continuation of our representative example of developing a c-Kit inhibitor, which is not based on the PD173955 scaffold, the above *in-silico* screening has produced the lead compound **AST-6536377** that is a member of quinoline-3-carbonitrile class of kinase inhibitors. A library of compounds based on this scaffold can be synthesized in three steps via parallel solution phase synthesis using the Advanced ChemTech Solution fully automated synthesizer as outlined (Scheme 1).



Treating 6,7-methylenedioxyquinoline-2-chloro-3-carbonitrile (**1**) with the appropriate Boc protected diamine (**2**) (with a variable spacer) in DMF under microwave conditions gives the 2-amino quinoline derivative (**3**). Note that the **first round of molecular diversity** is introduced with the variable spacer. Deprotection of the Boc protected amine (**3**) with TFA gives the primary amine (**4**) which is then treated with the appropriate isocyanate to give the final urea quinoline-3-carbonitrile derivative (**4**). Note that the **second round of molecular diversity** is introduced using a diverse set of isocyanates.

At this point we can select the appropriate candidates with high selectivity for the c-Kit,

and further optimize using molecular modeling for accommodation of  $^{124}\text{I}$ -label for PET imaging of c-Kit kinase activity while still retaining their therapeutic activity. An example of this methodology is outlined in scheme 2. Condensation of the primary amine (4) with the appropriate tributylstannyl isocyanate to give the tributylstannyl urea quinoline-3-carbonitrile derivative (5). Note, the **second round of molecular diversity** is introduced with the diverse set of isocyanates. The 4-tributylstannyl compound is then labeled using standard iododestannylation conditions with  $[^{124}\text{I}]\text{NaI}$ .



**Aim 3. Assessment of the biochemical characteristics of the focused library of c-Kit inhibitors** will consist of the following: (1) First we will study the c-Kit kinase inhibitory activity of cold (non-radiolabeled) analogues of newly synthesized derivatives using a commercially available purified recombinant c-Kit kinase (#7754, Cell Signaling Technology, In. MA). The enzymatic activity of c-Kit kinase will be measured using the c-Kit -specific tyrosine oligopeptide phosphorylation assay with ELISA readout of phosphorylated-peptide product buildup over time (Assay #7400; Cell Signaling Technology, Inc., MA). The assay conditions will be: 60 mM HEPES-NaOH, pH 7.5,  $\text{MgCl}_2$  3 mM,  $\text{MnCl}_2$  3 mM, Na-ortovanadate 3  $\mu\text{M}$ , DTT 1.2 mM, ATP (from 0 to 100  $\mu\text{M}$ ), 2.5  $\mu\text{g}/50\mu\text{l}$  of PEG 20.000, polyEY as the substrate 10  $\mu\text{g}/50\mu\text{l}$ , and IGF-IR 40  $\text{ng}/50\mu\text{l}$ . The assay protocol will be automated and performed on a custom-built robotic system EVO (Tecan, Switzerland) for high throughput and high content screening equipped with an ELISA washer and Sapphire fluorescence/spectrophotometry microplate reader. (2) From the high throughput screens a selection of hits will be made and ranked. The best hits will be further evaluated in a by screening against a pannel of different receptor and cytoplasmic tyrosine kinases (assays from Cell Signaling Technologies Inc.). (3) We will also directly measure the  $K_i$ , of the selected derivatives with high selectivity to c-Kit kinase using the recombinant c-Kit enzyme in presense of different concentrations of ATP and substrate peptide using an ELISA method as previously described.

**Assessment of antitumor efficacy *in vitro*.** We will study acute myeloid leukemia cell lines expressing different activity levels of c-Kit, including: HL-60, SKL-1. The level of

expression and activity of phospho-c-kit kinase and will be assessed in these cell lines by immunohistochemistry and quantified by ELISA. Compounds with highest specificity and activity against c-Kit will be tested in these cell lines. Each cell line growing in 96 well plates will be treated with different concentrations of selected compounds for three to four days. Tumor cell viability will be measured WST assay (Chemicon, Cat# 2210); cellular proliferation (DNA synthesis) will be measured using a classical  $^3\text{H}$ -thymidine incorporation assay; tumor cell apoptosis will be measured using fluorescence-based caspase-3 activity microplate assay (Molecular Probes, Cat #E-13184). The anti-tumor efficacy will be expressed in terms of IC<sub>50</sub> values for inhibition of viability, proliferation, and apoptosis, respectively. Specifically, we will compare these measures of anti-tumor efficacy of individual compounds with the levels of c-Kit and phospho-c-Kit in corresponding acute myeloid leukemia cell lines. Such an analysis should answer the question whether the level of expression and phosphorylation status of c-Kit could be used as a predictive marker of tumor sensitivity to the newly developed c-Kit inhibitors.

**Assess the anti-tumor efficacy of the selected c-Kit kinase inhibitors in engrafted immune compromised mice.**

In this specific aim, we will evaluate the *in vivo* anti-tumor efficacy of the selected c-Kit inhibitor with the highest potency against various tested cell lines determined *in vitro*. NOD/SCID mice will be engrafted with acute myeloid leukemia (AML) CD34+ cells in the method of Andreeff et al<sup>20</sup> and evaluated by southern blot and CD45 flow cytometric analyses. Three groups of animals (n=20) will start treatment with the selected the newly synthesized c-Kit inhibitors, which will administered p.o. BID in 60, 50, 35, or 25mg/Kg doses dissolved in 2ml of 90% water/10% DMSO per dose. Another group of animals (n=20) will be treated with 50mg/kg x2 BID p.o. of PD166326 dissolved in of 90% water/10% DMSO. The control group of animals (n=20) will be treated with of 90% water/10% DMSO in 2ml doses per animal. The proposed number of animals per study group (n=20) should provide statistically significant results because in the recent report by Ilaria et al<sup>21</sup> on the efficacy of PD166326 only 8 animals per group were sufficient to demonstrate efficacy of PD166326 *in vivo*. Therapy will be administered for two weeks during which tumor volumes and animal weights will be monitored to assess tumor growth inhibition and general toxicity of compounds at given dose and frequency. Plasma levels will be determined by automated LC-MS. We will evaluate the *in vivo* **biodistribution and tumor targeting of the selected radiolabelled c-Kit inhibitors** with the highest specificity and affinity to c-Kit kinase. using the same methodology of stated above. The monitoring growth will be performed using caliper measurements every other day. When tumors reach (~4-5 mm) in size for PET imaging of heterogeneity of c-Kit expression, they will be injected with one of the  $^{124}\text{I}$ -labeled c-Kit kinase radiotracer and imaged dynamically on a microPET scanner (Concord, TN). Thereafter, each animal will undergo a microCT scan (General Electric, IL) to anatomically co-register PET and CT images. During the course of dynamic PET imaging, arterial blood samples (from catheterized femoral artery) will be collected to analyze radiolabeled metabolites and to correct blood pool radioactivity measures derived from

the area of the heart on PET images. The arterial input function measures (and radiolabeled metabolite measures when possible) will be used for pharmacokinetic modeling (three-compartmental model, initially) and generation parametric images of c-Kit expression/activity in tumors.

Next, regional uptakes of selected radiolabeled c-Kit inhibitors will be measured by quantitative autoradiography and compared with immunohistochemically-determined phospho-c-Kit levels in corresponding regions of interest. Selected radiotracers will be assessed with respect to patterns of distribution and similarity to patterns of heterogeneity of c-Kit expression/activity. This analysis will be performed by quantitative autoradiography (QAR), which will be co-registered with the adjacent (or same) tumor sections that will be immunohistochemically stained for total c-Kit and phospho-c-Kit expression. These studies will be conducted using one tumor cell line with high and another with low c-Kit expression/activity (N=6 rats per tumor type x 2 tumor types x 2 radiotracers = 24 rats, total). By conducting these studies, we intend to assess two radiotracer(s) with the most optimal PK/PD and best specificity/sensitivity for imaging c-Kit expression/activity to be for further studies. In addition, it is our intention to complement this study with the dynamic tracking of human hematopoietic stem cell engraftment using in vivo bioluminescence imaging methodology of Crooks et al<sup>22</sup>. This should give an exact correlation of our radiotracer imaging to bioluminescence imaging.

**Assess the value of imaging c-Kit expression/activity as the inclusion criterion for therapy by c-Kit inhibitors** in multiple groups of animals bearing engrafted with acute myeloid leukemia (AML) CD34+ cells with different levels of c-Kit expression/activity by correlating imaging with the effectiveness of therapy with c-Kit-targeted drugs (newly synthesized c-Kit inhibitors and the previously described PD166326 as the reference compound). Three groups of mice bearing engrafted acute myeloid leukemia with high or low c-Kit expression/activity will be studied (20 mice for therapy with the newly synthesized c-Kit inhibitor, 20 rats for therapy with PD166326 and 20 for control group which will receive vehicle only; total = 60 rats). Tumor growth will be monitored by southern blot and CD45 flow cytometric analyses. When the tumor will reach 3-4mm in diameter, the animals will be imaged with PET using selected <sup>124</sup>I-labeled c-Kit kinase radiotracers. On the next day, the selected inhibitor will be administered at the most effective and tolerated dose as previously determined. The PD166326 will be administered at 50mg/kg x2 BID p.o. of (dissolved in 90% water/10% DMSO). Therapy will be administered 6 days a week for 2 weeks. The results of PET imaging of c-Kit expression and activity will be compared (post hoc analysis) with the efficacy of therapy to assess the value of imaging c-Kit expression and activity with PET for prediction of therapeutic efficacy of newly synthesized c-Kit inhibitors and compared to PD166326.

***In summary, conducting these studies we plan to develop and validate a novel class of molecular therapeutic agents targeted to c-Kit, Flt3, PDGFR $\alpha$ /PDGFR $\beta$  and c-Met kinase for the treatment of acute myeloid leukemia. Also we will evaluate c-Kit, Flt3, PDGFR $\alpha$ /PDGFR $\beta$  and c-Met and phospho- c-Kit, Flt3, PDGFR $\alpha$ /PDGFR $\beta$  and c-Met as tissue biomarkers of therapeutic efficacy for this class of compounds. Further more we will develop and validate novel radiolabeled agents for non-invasive***

***molecular imaging of c-Kit, Flt3, PDGFR $\alpha$ /PDGFR $\beta$  and c-Met expression/activity as biomarkers for prediction of tumor response to therapy.***

References

1. Pardan
- 2.
- 3.
4. Wisniewski D, Lambek CL, Liu C, Strife A, Veach DR, Nagar B, Young MA, Schindler T, Bornmann WG, Bertino JR, Kuriyan J, Clarkson B. Characterization of Potent
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1	40	—	0.70	350	170
1	120	150	0.1	1500	2100
1	40	—	0.28	275	780

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20. Monaco, G.; Lpmp[eav, M.; Munsell, M.; Leysath, C.; Wang, R-Y.; Jackson, C.E.; Korbling, M.; Estey, E.; Belmont J.; Andreeff, M., Engraftment of acute myeloid leukemia in NOD-SCID mice is independent of CXCR4 and predicts poor patient survival. *Stem Cells* **2004**, *22*, 188-201
21. Wolff, N.C.; Veach, D.R.; Tong W.P.; Bornmann W.G.; Clarkson, B.; Illaria, R.L., PD166326, a novel tyrosine kinase inhibitor, has greater anti-leukemic activity than imatinib in a murine model of chronic myeloid leukemia. (manuscript submitted to Cancer Research)
22. Wang, X.; Rosol, M.; Ge, S.; Peterson, D.; McNamara, G.; Pollack, H.; Kohn, D.B.; Nelson, M.D.; Crooks G.M., Dynamic tracking of human hematopoietic stem cell engraftment using in vivo bioluminescence imaging. *Blood***2003**, *102*, (10), 3478-3482