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Identification of novel candidate genes for targeted therapy of primary cutaneous T-cell lymphomas

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Primary cutaneous T-cell lymphomas (CTCL) represent a group of malignancies of mature T lymphocytes. The molecular mechanisms leading to CTCL are largely unknown and no curative therapy exists. To identify potential new targets for therapy, we analyzed the gene expression profiles in the most common subtypes, Mycosis fungoides (MF) and Sezary syndrome (SzS) with Affymetrix oligonucleotide microarrays, quantitative PCR and immunohistochemistry, in altogether 30 samples of lesional skin, blood and enriched CD4+ cells. The gene expression profiles were combined with findings of CGH of the same samples. For a third subtype, subcutaneous panniculitis-like T-cell lymphoma (SPTL), genomic DNA isolated from laser-microdissected malignant cells of seven cases was analysed with CGH and CGH on cDNA microarrays.

We identified a set of Th1-specific genes, like TBX21 (T-bet), to be downregulated in SzS and in some MF samples. In both SzS and MF blood cells, the S100P and the LIR9 gene expression was upregulated while IL7R and CD52 were upregulated in lesional skin. As for SPTL, genes likely to be involved in pathogenesis could be identified. For instance, DNA copy number losses were found on eight adjacent genes in the region 19p13.2−p13.1, among which are e.g. IL27R-alpha, essential to the initiation of Th1 response in T-helper cells, and the EGF-TM7 family membrane antigen CD97. DNA copy number gains were found in 3q26, frequently altered also in many solid tumors, and seven adjacent genes were found amplified, among which the proto-oncogene SNO and phospholipase D1. Also, losses in 12q were found in most cases of SPTL, which is in concordance with the identification of both DNA copy number loss and a downward gene expression bias in chromosome 12q found in the MF and SzS cases. This also matches with our recent finding of the CTCL-associated deletion/translocation of NAV3 (neuron navigator 3, 12q21) in all stages of CTCL.

Our findings thus revealed several new genes as potential targets for the development of specific diagnostic tools and targeted therapy of CTCL. Downregulation of a set of genes involved in Th1 polarization is for the first time associated with CTCL and regulation of T helper cell polarization seems to be crucial for the three most common subtypes of CTCL. Also, a plausible explanation for the proliferative response of CTCL cells to locally produced IL-7 was revealed.

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Identification and characterization of novel, orally active inhibitors of c-MET and Ron receptor tyrosine kinases

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The c-MET and Ron receptor tyrosine kinases have been implicated in many aspects of tumor biology, including tumor survival, angiogenesis, invasion and metastasis. Activation or overexpression of c-MET is also associated with the development and poor prognosis of a number of human cancers.

We have previously described our c-MET/VEGFR multitargeted kinase inhibitors. Here we describe a novel series of small molecule inhibitors with potent and selective activity against the c-MET and Ron receptor tyrosine kinases. Our c-MET/Ron selective molecules have IC50 values that ranged from 10−50 nM against recombinant c-MET and Ron receptors *in vitro*. Profiling in whole cells demonstrated that lead molecules potently inhibit c-MET and Ron receptor phosphorylation, c-MET and Ron dependent phenotypes such as HGF (c-MET ligand) and MSP (Ron ligand) stimulated cell scattering and migration of cancer cells. Importantly, these molecules potently suppressed the growth of tumor cells in which c-MET or Ron were activated. Pharmacokinetic evaluation of lead molecules demonstrated that the molecules are orally available and have favorable pharmacokinetics *in vivo*. Anti-tumor activity was dose dependent and correlated with inhibition of phosphorylation of target kinases as assessed by *in vivo* pharmacodynamic assays. The antitumor activity observed was not associated with body weight loss or marrow suppressive effects. These results show that our novel c-MET/Ron inhibitors are potent inhibitors of a unique set of kinases involved in cancer and have therapeutic potential for the treatment of cancer.

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SOD1 inhibition by tetrathiomolybdate demonstrates differential sensitivity against melanoma cell lines in vitro and in vivo: a possible method for identifying patients most likely to benefit from the second generation tetrathiomolybdate, ATN-224

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Background: Melanoma cells have been shown to be susceptible to cell kill and the induction of apoptosis by free radical generating agents *in vitro* and *in vivo*. ATN-224 is an inhibitor of the intracellular enzyme SOD1 and is currently being evaluated in several phase II trials. The inhibition of SOD1 decreases the flux of hydrogen peroxide and exposure to the drug for 48−72 hours leads to pleiotropic effects on cellular signaling. In this study, the sensitivity of a panel of melanoma cell lines to ATN-224 *in vitro* and *in vivo* was evaluated to assess rationale for a possible phase II clinical trial in melanoma.

Material and Methods: Several cell lines derived from different stages of disease in melanoma patients were evaluated including WM1205 (early primary disease), WM3211 (deep primary) and two cell lines (M-14 and SK-MEL-5) derived from metastases. *In vitro* proliferation was measured using Alamar Blue/MTT after exposing the cells to drug for 48−96 hours. *In vivo* studies were carried out using Balb-C *nu/nu* mice and injecting melanoma cells SC and staging the tumors to ~100 mm^3 prior to the initiation of treatment. ATN-224 was given once daily by gavage.

Results: ATN-224 had a significantly differential effect on melanoma cell proliferation *in vitro*. The most sensitive cell lines were the metastatic lines SK-MEL-5 and M-14 and cell kill was observed with an $IC_{50} \approx 5\,\mu M$. The early primary line WM1205 was very resistant to ATN-224 and complete cell kill was not achieved even at concentrations exceeding 100 μM whereas the deep primary line WM3211 was intermediate in its sensitivity. SK-MEL-5, M-14 and WM1205 are currently being evaluated for their *in vivo* sensitivity and these data will be presented.

Conclusions: Melanoma cell lines show profound differences in their *in vitro* sensitivity to ATN-224. If these differences translate to the *in vivo* situation, it may be possible to use an *in vitro* sensitivity assay to identify those melanoma patients that are most likely to benefit from ATN-224 treatment.

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An open-label study to characterize the pharmacokinetic (pk) parameters of erlotinib in patients with advanced solid tumors with adequate or moderately impaired hepatic function

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Epidermal growth factor receptor (EGFR) plays a critical role in proliferation, invasion and survival of tumor cells. Erlotinib (OSI-774, Tarceva®) is an oral, reversible inhibitor of the EGFR (HER1/EGFR) tyrosine kinase. Erlotinib (E) is primarily metabolized by hepatic cytochrome P450 isoforms. A prior study demonstrated reduced E clearance and recommended dose reduction in patients (pts) with hepatic dysfunction. The primary objective of this study is to compare PK parameters of E in pts with adequate hepatic function (AHF) and moderate hepatic impairment (MHI). In this open-label study, 42 pts (21 AHF and 21 MHI) were to receive a single dose of E 150 mg on day 1 followed by plasma samples for PK and protein binding (PB) studies over a 96-hour period. All cohorts required nonsmoking pts with advanced solid tumors potentially responsive to E or for which no effective therapy was available. AHF was defined as Tbili $\leqslant$ ULN and AST/ALT $\leqslant 1.5 \times$ ULN, whereas a Child-Pugh score of 7–9 constituted MHI. Levels of E and metabolite, OSI-420, were determined by a validated LC/MS/MS method and PK parameters were calculated by noncompartmental analysis. Plasma PB of E was determined by an ultracentrifugation technique. To date, 29 pts have been enrolled: 21 AHF and 8 MHI. Enrollment into the AHF cohort is complete. Demographic data are available for 23 pts: 16 AHF and 7 MHI. The AHF cohort includes 8 females and 8 males with a median (M) age of 63 (40−85) and M PS of 1 (0–2). The MHI cohort includes 2 females and 5 males with a M age of 55 (46−66) and M PS of 1 (1–2). All pts were evaluable for PK analysis and toxicity (tx). Preliminary PK data are available for 19 pts (13 AHF

and 6 MHI). Following a single dose of E: M C_{max} 1.16 (0.620–1.87) vs 1.28 (0.241–2.35) μg/mL, T_{max} 2 (1–6) vs 6 (2–8) hours, M AUC_{0-inf} 30.9 (21.0−79.5) vs 41.5 (4.71−80.5) μg hr/mL, and M clearance 4.86 (1.89–7.15) vs 3.88 (1.86−31.9) L/hr in the AHF and MHI cohorts respectively. Plasma PB data available for 10 pts (7 AFH and 3 MHI) shows the percent of E bound is 97.3 (96.6−98.2) in AHF vs 97.2 (96.2−98.0) in MHI. Clinical data are available for 22 pts (16 AHF and 6 MHI). One MHI pt discontinued study prior to starting drug and is not included in the safety discussion. Common grade 1–2 adverse events related to E include diarrhea [4 (25%) vs 1 (17%)], nausea [6 (38%) vs 2 (33%)], acneiform rash [3 (19%) vs 2 (33%)], anorexia [4 (25%) vs 1 (17%)] and fatigue [4 (25%) vs 0 (0%)] in the AHF and MHI cohorts respectively. In the AHF cohort, 3 pts (19%) experienced tx related to E, including 1 pt (6%) with grade 3 diarrhea and 2 pts (13%) with grade 3 acneiform rash. No grade 3 related tx have been reported in the MHI cohort. There have been no grade 4 tx or serious adverse events related to E reported in either cohort. Preliminary data suggests that MHI may increase systemic exposure to E with no effect on plasma PB and drug-related tx. Enrollment into the MHI cohort is ongoing. Updated information will be presented.

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Discovery and characterization of a series of Axl kinase inhibitors using the CLIMB process

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Axl is a unique receptor tyrosine kinase, which has been implicated in inhibition of apoptosis, especially through counteraction of the pro-apoptotic activities of E1A, and is emerging as a viable target for a number of human malignancies, both solid and hematological. This protection is believed to operate through the action of Src and PI3K (via the Akt pathway), which are known proto-oncogenes themselves. Expression of Axl and its substrate, Gas6, has been implicated in defense against apoptosis in a variety of tumor subtypes. Overexpression of Axl in cells of the myeloid lineage also leads to a non-insulin dependent diabetes mellitus-like, or Type II diabetes phenotype. For these reasons, it is clear that there is an unfilled need for small molecule inhibitors of Axl kinase in the clinic, and we have set out to create such an entity. We have developed a proprietary drug discovery process, called CLIMB™, which we utilized for the identification of novel Axl modulating compounds with therapeutic potential. In traditional small molecule screening, as many as several million compounds may be tested in order to identify the few that interact selectively with a disease-related protein target. CLIMB™ can achieve similar results by screening as few as several hundred computationally selected compounds. CLIMB™ screening is based on the clustering of representative chemical structures and pharmacophores that embody our large virtual library of nearly 50 million compound structures. A homology model for Axl kinase was built using the known crystal structures for insulin-like growth factor-1 receptor and c-Met tyrosine kinases, and subjected to docking with an expansive virtual library of in-house and commercially available compounds. After passage through a series of in silico filters designed to predict pharmacological and physicochemical parameters, the “most drug-like” candidates with favorable predicted binding energies were selected for further biological and biochemical testing. Several compounds resulting from the computational screen bore significant activity (low micromolar to nanomolar) against recombinant Axl protein in an in vitro assay, validating the effectiveness of the CLIMB™ process in reducing time and cost of early lead identification. These compounds also demonstrate potent cell-based activity in a variety of tumor cell lines and in xenograft animal models, making them promising anti-cancer therapeutic leads.

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Discovery and characterization of a small molecule inhibitor for pim-1 kinase

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The Pim-1 serine/threonine kinase is known to be involved in a number of cytokine signaling pathways as a downstream effector. Once activated, Pim-1 kinase causes progression of the cell cycle, inhibition of apoptosis, and modulation of other signal transduction pathways, including its own. Pim-1 kinase is also shown to effect activation of transcription factors like NFAT, p100, c-Myb, and Pap-1 and inhibition of others such as HP1. Normal expression of Pim-1 kinase is seen in cells of hematopoietic origin, such as fetal liver, thymus, spleen, and bone marrow, additionally expression is also seen in prostate and oral epithelial cells. Pim-1 kinase is believed to be involved in the initiation or progression of malignant transformation leading to malignancies including Burkitt’s lymphoma, prostate cancer, oral cancer, and diffuse large cell lymphomas, among others. We have identified a series of Pim-1 kinase inhibitors, based on a heretofore-unexploited pharmacophore, using our proprietary CLIMB™ drug discovery process. Through the use of CLIMB™, the published Pim-1 kinase crystal structure was used as a substrate for docking of a very large virtual library, composed of in-house and commercially available small molecules, to generate a subset of leads based on calculated binding energies. These leads were then screened using a number of in silico physicochemical and ADMET prediction algorithms to determine “druggable” leads which were most likely to be successful in a biological context. Lead candidates were initially screened using biochemical enzyme-based or cell-based assays. Cell-based activity was determined in Panc-1 (pancreas), A549 (non-small cell lung), and PC-3 (prostate) cancer cell lines. At an initial concentration of 100 micromolar numerous candidates inhibited cell growth by over 60% compared to untreated controls in a preliminary screen. In the Pim-1 in vitro kinase assay two candidates exhibited inhibitory activity with IC_{50} concentrations in the low micromolar range. The lead candidates for pim-1 kinase inhibitors discovered through the CLIMB™ process have shown good biochemical and biological activity, based off of physical screening of less than 100 compounds, chosen from a library of millions, each of which show activity at a considerable level. Based off of the two most promising lead candidates a series of analog candidates are currently being produced to refine inhibitory activity and pharmacokinetic characteristics.

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Discovery and characterization of novel small molecule inhibitors of polo-like kinase-1, using a computational development process

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Polo-like kinases belong to a family of well-conserved kinases found throughout the eukaryotes. In humans, high levels of polo-like kinase-1 (Plk1) have been associated with poor prognosis in numerous cancer types including breast, colon, non-small cell lung cancer, and the difficult-to-treat pancreatic cancer, along with others, and has been validated as a target in these tumor types. Polo-like kinase-1 is a mitotic serine-threonine kinase which plays a very important role in the processes of centrosome separation, spindle formation and sister chromatid segregation. As well, it drives progression through the G2/M checkpoint by virtue of phosphorylation of Cdc25c, leading to an active Cdk1/CyclinB1 complex. RNA interference has shown that disruption of Plk-1 leads to such effects as mitotic arrest, cytokinetic failure and induction of apoptosis. Using our proprietary CLIMB™ drug discovery process, we have identified and synthesized a series of polo-like kinase-1 inhibitors. Using the Cdk1 crystal structure as a backbone, a homology model of polo-like kinase-1 was built and subjected to virtual docking algorithms in the context of a large in-house virtual collection of small molecules from a diversity of sources. Through the employment of several in silico predictors, compounds with strong binding interactions were ranked according to calculated ADMET properties and chemical characteristics (including solubility, partition coefficient, expected permeabilities and physical properties) before any in vitro assays were undertaken. This has allowed us to remove “non-druggable” leads before time and resources are wasted on development of undesirable compounds. Screening of lead candidates for inhibitory activity using the Z′-LYTE biochemical assay, which measures phosphorylation of a serine or threonine residue on a synthetic FRET-peptide by recombinant polo-like kinase-1, demonstrated that multiple lead candidates exhibited IC_{50} activities below 10 μM for the inhibition of polo-like kinase-1. This is significant, given that relatively few (less than 75) compounds from the computational screens have been tested, and all bear activity to some extent against the recombinant target. Cell-based testing on tumor cells also revealed considerable activity in cell culture. Analogs of these initial leads have been synthesized to improve their activity and specificity, giving way to a series of preclinical candidates for the treatment of a variety of cancer diseases.

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Identification of molecular targets in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the major histological type of primary liver cancer. HCC is the fifth most common cancer and the third leading cancer killer worldwide, and is responsible for about half million new cases and almost as many deaths per year. Surgical resection in the form of partial hepatectomy or liver transplantation is the mainstay for curative treatment. However, only 20% of all patients are eligible for surgery because the majority of patients are diagnosed at advanced stage with intra- and/or extra-hepatic metastasis. Nonetheless, recurrence is still common after curative surgery with approximately 50% at 5-year. Prognosis