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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