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Efficacy of the novel multi-targeted kinase inhibitor JNJ-26483327 in experimental brain and bone metastases models

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The clinical success of imatinib, gefitinib and erlotinib have demonstrated the potential of small-molecule kinase inhibitors in cancer therapy. Antibody based approaches (trastuzumab and cetuximab) have also proved highly successful in targeting the HER family of kinases. However, there is emerging evidence that patients who respond to these agents still develop brain metastases probably due to the documented poor penetration of the available agents through the intact blood brain barrier. There is clearly a need for similar agents that can cross the intact blood brain barrier and thereby inhibit the development of micro-metastases in this privileged site. JNJ-26483327 is a novel orally active macrocyclic multi-targeted kinase inhibitor (MTKI) that has a unique kinase inhibition profile (pan-Her family, Src family and VEGFR3 inhibition) and demonstrated potent anti tumour activity in a number of subcutaneous xenograft models. The unique physico-chemical properties of JNJ-26483327 has resulted in an extremely favourable tissue distribution profile including the ability to cross the intact blood brain barrier whilst still retaining good cellular activity and oral bioavailability. Here, we describe the unique pre-clinical biodistribution profile of JNJ-26483327, that demonstrates high (relative to plasma levels) levels of the compound in a number of organs known to be predisposed to the development of primary or metastatic tumours. We further demonstrate that this preferential tissue distribution to the brain and bone marrow compartment results in significant anti tumoural activity using experimental models of brain and bone metastases.
Our data demonstrate that JNJ-26483327 potently delays tumour growth, leading to increased survival in experimental models of brain metastases and a significant reduction of bone destruction due to inhibition of tumour growth within the bone environment. In summary the data indicates that this compound, currently undergoing clinical testing, has the potential to outperform tyrosine kinase inhibitors currently in clinical use.

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JNJ-26483327 is a novel multi-targeted tyrosine kinase inhibitor with cellular activity against EGFR, Her2, Src and VEGFR3

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The clinical success of imatinib, gefitinib and erlotinib have demonstrated the potential of small-molecule kinase inhibitors in cancer therapy. However, the emergence of resistance and the low patient response rates are important problems associated with these compounds, which can be addressed through the development of multi-targeted kinase inhibitors.
Here, we describe the characterization of a novel, small-molecule, multi-targeted tyrosine kinase inhibitor, JNJ-26483327. *In vitro* kinase assays indicate that JNJ-26483327 is a potent inhibitor of EGFR family members (EGFR, Her2 and Her4), Src family kinases (Lyn, Yes, Fyn, Lck and Src) and the receptor tyrosine kinase VEGFR3. In EGFR-driven squamous carcinoma cells (A431), grown as a mouse xenograft, orally dosed JNJ-26483327 inhibited EGFR autophosphorylation (at Y1068 and Y845) and phosphorylation of several effectors (STAT1/3, Akt and PLCγ), resulting in inhibition of tumour growth. In the non-small cell lung cancer cell lines H3255 and H322, EGFR and Her2 autophosphorylation are potently inhibited by JNJ-26483327, resulting in inhibition of cell proliferation. Also the Her2-driven breast cancer cell line BT474 is highly responsive to JNJ-26483327 in a proliferation assay. Her2, Erk1/2 and Akt phosphorylation are all inhibited in BT474 cells treated with JNJ-26483327. In addition, BT474 cells round up, detach and induce apoptosis (as measured by cleavage of PARP and Caspase-3), all within 6 hours after compound addition. The selective EGFR/Her2 inhibitor lapatinib, but not erlotinib, also induced rounding and detachment of BT474 cells, albeit to a lesser extent then JNJ-26483327. Intriguingly, lapatinib-induced detachment resulted in increased autophosphorylation of Src at Y416, whereas JNJ-26483327 induced cell detachment did not. The increase in Src autophosphorylation in lapatinib-treated detached cells was prevented by simultaneous incubation with the Src inhibitor PP2. In support of the ability of JNJ-26483327 to inhibit Src, independent of EGFR inhibition, we found that JNJ-26483327 abrogated Src-dependent PDGF signaling in mouse 3T3 cells whereas lapatinib and erlotinib did not. JNJ-26483327 activity against VEGFR3 was confirmed in human vascular endothelial cells (HMVECd), in which JNJ-26483327 prevented VEGFR3 dependent VEGF-C stimulation of Erk1/2, whereas it did not affect VEGFR1 mediated VEGF signaling.
In summary, our data demonstrate that JNJ-26483327 is a potent tyrosine kinase inhibitor targeted at Her and Src family kinases as well as VEGFR3. The unique spectrum of these targets, relevant at various stages of cancer development (cell proliferation, invasion, migration and metastasis), suggest this compound, currently in clinical trials, has the potential to outperform tyrosine kinase inhibitors currently in clinical use.

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Alteration of the RAS/BRAF/MEK/MAPK pathway by BAY 43–9006 (sorafenib) in metastatic melanoma

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Background: Sorafenib is a multikinase inhibitor. The *RAS*/*BRAF*/*MEK*/*MAPK* pathway (pw) is a major pw for transmitting extracellular growth factor signals to the nucleus. Approximately 60% of melanomas carry B-Raf mutations. The primary trial goals are: (1) determine if sorafenib can disrupt the *RAS*/*BRAF*/*MEK*/*MAPK* pw signaling; and (2) identify differential anti-tumor activity in melanoma pts whose tumors have been molecularly characterized.
Methods: Biopsy (bx)-accessible, untreated metastatic melanoma. Measurable disease (RECIST). Adequate hematologic and serologic parameters. Signed ICF. All pts had tumor bx for B-Raf status determined by PCR sequencing of Exon 3 of N-Ras and exon 15 of B-Raf prior to tx. Pts were stratified by B-Raf. Tx: BAY 43–9006 was administered at 400 mg po BID. D1−28 q4w. Repeat bx was done on Day 28. Pre- and post-bx were assessed for B-Raf, N-Ras, ki-67, cyclin-D1 and pERK. Collagen cryptic epitopes were measured serially to allow assessment with response. Re-imaging was done every 2 cycles and pts treated until POD. Stats: 2 stage study design with accrual of 13 pts/arm in first stage and total of 29 pts/arm if $\geqslant$1 objective response (OR) in initial cohort.
Results: 19 pts (5-M1a, 4-M1b, 10-M1c) enrolled. 17 wild type (WT) and 2 mutant (M) B-Raf. Median age: 54 (range 23−92). 4 pts with LDH $\geqslant 1.5 \times$ nl. Responses: 6 NE (1 WD, 5 early POD), M B-Raf -1 PR (lymph nodes and large SQ arm masses) and 1 PD; WT B-Raf-1 PR, 5 POD after 2 cycles, 3 SD, 2 too early. *Toxicity*: Grade I diarrhea, Grade II hypertension, fatigue and oral mucositis, Grade III hand-foot syndrome. Matched paired biopsies on all pts demonstrated down regulation of tumor ki-67, erk and cyclin-D1. Clinical responses were seen in both arms. The levels of the serum novel collagen cryptic epitopes correlates with tumor responses.
Conclusions: This Phase II trial has molecularly characterized tumors for B-Raf status prior to tx with sorafenib and assessed the effects on Ras-Raf signaling and correlates clinical responses. While accrual to the study is still ongoing, responses include 1 pt with M B-Raf and 1 pt with W B-raf have had an OR (PR) with a significant decrease in tumor volume. Correlative studies support clinical responses.
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Simultaneous inhibition of IGF1R and Src family kinases causes tumor growth inhibition and tumor regression in xenograft models

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Insulin-like growth factor 1 receptor (IGF1R) and Src play important roles in tumor cell proliferation, survival, and metastasis. EXEL-2280 (XL228) is a potent small molecule inhibitor of these kinases, with low nanomolar potency in biochemical assays. In cell-based assays, EXEL-2280 inhibits ligand-induced autophosphorylation of IGF1R and of insulin receptor (IR), and downstream phosphorylation and activation of IRS-1 and AKT. In addition, EXEL-2280 inhibits Src-dependent phosphorylation of focal adhesion kinase (FAK) in multiple tumor cell lines.
In pharmacodynamic (PD) studies in nude mice, oral administration of EXEL-2280 results in the inhibition of phosphorylation of IGF1R and the downstream signaling kinase AKT in xenograft tumors. EXEL-2280 also inhibits Src-dependent phosphorylation of FAK in tumors, and exhibits a sustained duration of action in PD studies with inhibition >50% up to 7 h after a single oral dose. EXEL-2280 exhibits significant anti-tumor activity in MCF7, Colo-205, HT29, and A549 human tumor xenograft models, with substantial tumor regression observed in the MCF7 and Colo-205 models with once daily or less frequent oral dosing. Immunohistochemical analyses of tumors taken at the end of efficacy studies reveal that EXEL-2280 causes decreased tumor cell proliferation and increased tumor necrosis/apoptosis. Overall, these data demonstrate that administration of EXEL-2280 inhibits IGF1R and Src in vivo, and results in cytoreductive anti-tumor efficacy in multiple xenograft models. These results provide a rational basis for the clinical development of EXEL-2280 for the treatment of solid tumors.