NT-113, A Novel, High CNS Penetrance pan-ERBB Inhibitor for Glioma Therapy

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We reported a novel, high CNS (central nervous system) penetrance pan-ERBB inhibitor, NT-113, for anti-glioma therapy. Using five intracranial glioblastoma (GBM) models, we found that NT-113 significantly suppresses growth of tumors overexpressing wild-type EGFR or the EGFRvIII mutant, resulting in improved animal survival. NT-113 inhibits ERBB activity at low nanomolar (< 5 nM) concentrations, and suppresses its downstream signaling through Akt, leading to reduced cell proliferation and increased apoptosis. When compared with previous generations of EGFR small tyrosine kinase inhibitors (TKIs), erlotinib and lapatanib, NT-113 shows the most substantial effect on tumor growth inhibition and animal survival. The superior anti-glioma activity of NT-113 can be explained by broader spectrum of ERBB inhibition, efficient brain penetrance, and superior tumor uptake. Our data support clinical investigation of NT-113 in glioma over-expressing wild type or vIII forms of EGFR.


The ERBB family proteins include four receptor tyrosine kinases: EGFR (ERBB1), ERBB2, ERBB3 and ERBB4. ERBB receptors are structurally similar, consisting of an extracellular ligand binding domain, a transmembrane region and an intracellular tyrosine kinase domain. EGFR, ERBB3, and ERBB4 respond to ligand by inducing receptor homodimerization or heterodimerization, whereas ERBB2 has no identified ligand and functions as the preferred ERBB dimerization partner [1]. Upon ligand binding, EGFR undergoes a conformational change that promotes receptor dimerization, resulting in auto-phosphorylation of the intracellular domain, which in turn elicits downstream signaling through multiple pathways, including MAP kinase and PI3K/Akt pathways. EGFR signaling plays an important role in supporting cell growth, proliferation and survival.

Epidermal growth factor receptor (EGFR) mutation and amplification occur frequently in human cancer, especially glioblastoma multiforme (GBM; WHO Grade IV), the most common and malignant form of primary brain tumor in adults [2]. EGFR amplification and mutation is found in 40% of adult GBM [3,4], making it an attractive therapeutic target in a disease where chemotherapy has been largely ineffective, in part because of poor CNS distribution of systemically administered therapy.
EGFR inhibitors have been developed and approved by the FDA for breast (lapatinib), non small cell lung cancer (afatinib; erlotinib; gefitinib) and pancreatic cancer (erlotinib), but showed disappointing results in clinical trials of glioma [5-7]. Two classes of EGFR inhibitors have been tested in clinical trials: monoclonal antibodies that act on the extracellular domain of EGFR, thus preventing ligand binding; and small molecule tyrosine kinase inhibitors (TKI) that target the intracellular tyrosine kinase domain and prevent receptor autophosphorylation and activation.

A number of EGFR TKIs have been investigated for their efficacy in treating GBM. First generation EGFR TKIs, such as erlotinib (OSI-774; Tarceva; OSI Pharmaceuticals) and gefitinib (ZD1839;Iressa; AstraZeneca) are reversible EGFR inhibitors that competitively bind to ATP-binding sites, preventing auto-phosphorylation and consequently inhibiting downstream signaling. Gefitinib has shown limited efficacy on GBM as single agent [8]. Similarly, disappointing clinical outcome has been documented for erlotinib in GBM [7]. To improve small molecule inhibitor efficacy, second generation TKIs have been developed to target multiple ERBB family members, thereby interfering with ERBB activation through hetero- and well as homo-dimerization. An example is the reversible TKI lapatinib (GW572016; Tykerb, GlaxoSmithKline) that has dual activity towards EGFR and ERBB2. Despite the logic associated with its use in treating GBM, lapatinib has shown limited anti-tumor activity in clinical trials for this cancer [6], and despite initial response of lung cancer to reversible ERBB TKIs, acquired resistance to sustained TKI treatment has prompted the development of third-generation TKIs that block kinase activity via covalent bonding, allowing prolonged, irreversible receptor inhibition [9]. Third generation TKIs usually have broad spectrum activity against multiple receptors in the ERBB family, and may be more efficient against mutant receptors that are relatively unresponsive to first generation TKIs. Third generation TKIs include afatinib (BBW 2992; Gloritif; Boehringer Ingelheim Pharmaceuticals), neratinib (HKI-272; Puma Biotechnology) and dacomitinib (PF-00299804; Pfizer). Afatinib, which targets EGFR and ERBB2, has shown limited activity in recurrent GBM when used as a single agent [10]. The pan-ERBB inhibitor dacomitinib (clinical trial # NCT01441596) [11] and the EGFR-ERBB2 inhibitor neratinib (clinical trial # NCT01494662) [12] are under phase II clinical trial for effectiveness in treating breast cancer patients suffering from brain metastases and breast cancer derived brain metastases respectively. We have recently published pre-clinical results for another irreversible, orally-administered pan-ERBB inhibitor, NT-113, when used in treating mice with intracranial GBM xenografts tumor [13].

Using five GBM xenograft models, we showed that NT-113 efficiently inhibits intracranial tumor growth and improved survival in animals bearing wild type EGFR and especially EGFRvIII amplified glioma. In each of the three EGFRvIII amplified GBM models (GBM39, GBM6, U87: EGFRvIII) and a wild type EGFR amplified model (GBM12), NT-113 (10 mg/kg/day) significantly reduced intracranial tumor growth rate and extended animal survival. Ex vivo immunoblotting analysis reveals that NT-113 inhibits EGFR phosphorylation and downstream Akt signaling. Moreover, tumors treated with NT-113 showed reduced cell proliferation and increased apoptosis as indicated by deceased Ki-67 and elevated cleaved caspase 3 staining, respectively.

Amplification and overexpression of EGFRvIII in GBM has been associated with tumor responsiveness to EGFR inhibition [14,15]. To explore the relationship between EGFR expression status and NT-113 response, we used an isogenic GBM cell pair U87-U87: EGFRvIII. U87: EGFRvIII is a derivative of the parental U87 cell line generated by retroviral EGFRvIII modification. We found that NT-113 treatment significantly reduces intracranial U87: EGFRvIII growth, resulting in extended animal survival, whereas parental U87 xenografts are unresponsive to NT-113 treatment. Collectively, our data suggests that patients with gliomas expressing amplified wild type or the vIII form of EGFR are candidates for NT-113 treatment.

We also showed that NT-113 out-performed erlotinib and lapatinib in reducing tumor size and extended animal survival in an intracranial model with amplified wildtype EGFR (GBM12). Concordant with our in vivo finding, immunoblot analyses of GBM cell lines showed that at 1µM concentration, NT-113 results in the most significant suppression of EGFR phosphorylation and downstream Akt activation. We concluded that the superior anti-GBM activity of NT-113 is likely due to a combination of (i) high potency, (ii) pan-ERBB activity, and (iii) superior CNS biodistribution.

ERBB receptors function in concert to transmit signals, and preclinical data indicates that the most efficient ERBB-targeted strategies are those that inhibit multiple ERBB receptors [16]. We showed that NT-113 inhibits EGFR, ERBB2 and ERBB4 at low nano molar (< 5 nM) concentrations, and that IC50 values of NT-113 for ERBB family proteins are significantly lower in comparison to most third generation ERBB TKIs, including afatinib, dacomitinib and neratinib (Table 1), suggesting superior NT-113 potency.
Blood brain barrier penetrance remains a challenge in small molecule based anti-glioma therapy, and whereas first generation TKIs erlotinib and lapatinib have demonstrated some efficacy against peripheral solid tumors, these agents have been mostly ineffective in GBM clinical trials, very likely because of poor CNS access \[21,22\]. We showed that NT-113 has significantly higher blood brain barrier penetrance compared to erlotinib, as indicated by a 17 fold higher brain to plasma ratio for NT-113 compared to erlotinib, with each agent administered orally (brain to plasma drug concentration of erlotinib vs NT-113 are 0.01 and 0.17 respectively), and even though animals were treated with 10 fold higher dosage of erlotinib than NT-113 (100 vs. 10 mg/kg/day). Further analysis of tumor drug concentration revealed that NT-113 is preferentially sequestered in tumor (tumor to normal brain ratio = 10.5). Collectively, our data suggest that NT-113 exhibits high CNS penetrance and preferential uptake by tumors.

A major concern for TKI monotherapy in glioma is acquired drug resistance through adaptive mechanisms \[23,24\]. For two of the GBM models tested, complete tumor stasis was achieved when treating GBM39 (EGFRvIII amplified) with NT-113 over a two week period, whereas GBM12 (wild type EGFR amplified) showed some degree of adaptation to NT-113 in relation to two EGFR TKIs, erlotinib and lapatinib, that have seen substantial use in clinical practice. We demonstrated the potential of NT-113 for treating EGFR wild type and vIII amplification GBM, and possibly other ERBB-driven cancers as well.

Our in vitro and preclinical results show superior activity of the novel pan-ERBB inhibitor NT-113 in relation to two EGFR TKIs, erlotinib and lapatinib, that have seen substantial use in clinical practice. We demonstrated the potential of NT-113 for treating EGFR wild type and vIII amplification GBM, and possibly other ERBB-driven cancers as well.

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Table 1. Comparison of IC<sub>50</sub> of ERBB family for EGFR TKIs

<table>
<thead>
<tr>
<th>TKIs</th>
<th>EGFR</th>
<th>ERBB2</th>
<th>ERBB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; generation</td>
<td></td>
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<tr>
<td>irreversible pan-ERBB</td>
<td></td>
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<tr>
<td>NT-113 [15]</td>
<td>0.4</td>
<td>4.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Afatinib [17]</td>
<td>0.5</td>
<td>14</td>
<td>NA</td>
</tr>
<tr>
<td>Dacomitinib [19]</td>
<td>6</td>
<td>45.7</td>
<td>73.7</td>
</tr>
<tr>
<td>Neratinib [19]</td>
<td>92</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; generation</td>
<td></td>
<td></td>
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<tr>
<td>reversible EGFR, ERBB2</td>
<td></td>
<td></td>
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<tr>
<td>Lapatinib [25]</td>
<td>10.8</td>
<td>9.2</td>
<td>347</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; generation on market</td>
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<tr>
<td>reversible EGFR</td>
<td></td>
<td></td>
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<tr>
<td>Erlotinib [11]</td>
<td>0.56</td>
<td>512</td>
<td>790</td>
</tr>
<tr>
<td>Gefitinib [11]</td>
<td>3.1</td>
<td>343</td>
<td>476</td>
</tr>
</tbody>
</table>

References

11. Boehlering Ingelheim. Lux-breast 3; randomised phase II study of afatinib alone or in combination with vinorelbine versus investigator's choice of treatment in patients with HER2 positive breast cancer with progressive brain metastases after trastuzumab


