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Title: **Revoking the Privilege: Targeting HER2 in the CNS**

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Running title: **Targeting Her2 in the CNS**

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Abstract:

Pharmacologic agents developed for cancer therapy have traditionally relied on a therapeutic ratio of effects between tumors and normal tissue. Over the past decade this concept has been refined through the development of agents that are intended to specifically target tumor cells. The EGFR (ErbB) family of receptor tyrosine kinases is an intensely studied target in many cancer cell types and several successful therapeutic agents have been developed to block the growth promoting functions of these receptors. However, with their success has come the evolution of novel clinical scenarios by which tumor cells can evade specific therapies. Trastuzumab, a monoclonal antibody to Her2/ErbB2 that is utilized in breast cancer, has been shown to provide a survival benefit for patients whose tumors express this receptor, but does not have activity in the central nervous system due to the blood brain barrier. Efforts to improve current strategies of targeting this receptor may lead not only to benefits in the treatment of breast cancer but also to advances in the treatment of other CNS malignancies such as gliomas and medulloblastoma.

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In 1906 speaking about the treatment of bacterial pathogens Paul Ehrlich remarked that it will "be easy to effect a cure if substances have been discovered which have a specific affinity for these bacteria and act…on these alone. . . while they possess no affinity for the normal constituents of the body. . . such substances would then be . . . magic bullets." Ehrlich's scientific work was subsequently awarded the Nobel Prize in 1908. With the advent of molecularly targeted therapies in oncology has come the promise of "magic bullets" in the treatment of malignancy. We now have agents that enable disease specific treatment with reduced normal tissue toxicity. However, as more and more molecularly targeted agents enter clinical evaluation we are seeing that finding a magic bullet does indeed take a certain amount of magic. The utility of a targeted agent within a patient population will depend upon the appropriate answers to a number of questions.

First, is the molecular target present within the tumor?

Second, can one "hit" the target with the agent of interest?

Third, is there some clinically meaningful end-point as a result of the specific inhibition of the target in this disease?

In the case of Imatabib (Gleevec) the initial results were resoundingly "Yes" on all accounts. The BCR-ABL tyrosine kinase was present and active in virtually all CML cells, the tyrosine kinase was accessible and specifically inhibited by the drug, and clinical remissions were observed in 50% of patients using a single-agent even when in blast crisis(Druker et al., 2001a; Druker et al., 2001b). Truly a remarkable achievement that would have made Ehrlich proud! Initial enthusiasm for gefitinib for the treatment of

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non-small cell lung cancer was also high as 40-80% of non-small cell lung cancers over-express EGFR (the target for gefitinib and erlotinib) and dramatic responses were observed in animal models and initial clinical studies(Kris et al., 2003). Only later was it determined that EGFR was present, biologically active, and critical in only a sub-set of patients thus yielding dramatic responses in a small number of patients (often 10-20%) with no response in the bulk of the remainder(Lynch et al., 2004). Here was an example of the target only being present in a fraction of all patients and being biologically relevant in an even smaller population. Therefore, applying the "rules" described above one clearly can find a population of patients who express the target and one may even be able to block its activity; however, the impact of this inhibition may or may not have clinical relevance. The magic in this case is deciding exactly who will benefit from this particular bullet.(Sordella et al., 2004)

For trastuzamab (Herceptin) in the treatment of Her2/neu positive breast cancer yet another twist appeared in this interplay between biologic characteristics of the cancer and pharmacokinetics of the therapeutic agent. Results to date have been particularly encouraging as Her2/neu is clearly over-expressed and plays a significant role in a sizable sub-population of patients with breast cancer. In addition, treatment with trastuzamab inhibits the activity of this critical pathway resulting in clinically astounding improvements in disease free and over-all survival(Piccart-Gebhart et al., 2005; Romond et al., 2005). However, only as time has past have we begun to observe the limitations in this particular targeted agent. The first hint of the treatment hitting more than the targeted cells was focused on the presence of Her2/neu in cardiac myocytes and thus a

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resulting decrease in cardiac function particularly when combined with anthracyclines. With time it appears that this limitation may not be as concerning as once was thought(Bria et al., 2007; Ewer et al., 2005). However, now a new clinical limitation has been observed with a sizable portion of women with Her2/neu positive breast cancer who are otherwise free of disease. These women now present with metastatic disease to the brain (Bria et al., 2007; Piccart-Gebhart et al., 2005; Romond et al., 2005), a 'sanctuary' or 'privileged' site from many systemic therapies. Falling back again to the guidelines described above we can see that although present and biologically active Her2/neu may not always be "hit" by trastuzamab. In this case the very nature of the agent as an antibody (see **figure 1**), with high molecular weight, enables high selectivity for Her2/neu but also limits its penetration past the blood-brain-barrier (BBB). (Coincidently, the first observations of the existence of the BBB were described by Ehrlich). This inability to target tumor cells isolated in the CNS presumably led to the newly identified clinical phenomena where a larger portion of women with Her2/neu positive disease are relapsing only in the CNS following trastuzamab treatment; a change in the natural history of breast cancer.

In this issue of Molecular Pharmacology, Emanuel and colleagues have identified a novel compound (JNJ-2871063) that is an inhibitor of the ErbB family of receptor tyrosine kinases (RTK). In this study the investigators analyzed kinase activity, receptor phosphorylation status, and results from high throughput kinase profiling to demonstrate the specificity and effectiveness of this new compound on the family of ErbB receptors. The comparisons made between the JNJ compound and other ErbB RTK inhibitors

underscore the fact that in general tyrosine kinase inhibitors have multiple targets and cellular effects that are unpredictable and thus require empiric demonstration of efficacy in the clinical setting. Yet the study presented by Emanuel et. al. suggests that if the ErbB RTK family is the target for anti-cancer therapy, then this compound may have both enhanced selectivity and efficacy compared to previous small molecule RTK inhibitors. The second notable finding of JNJ-2871063 is its activity in the central nervous system, a site privileged from both trastuzamab and possibly other small molecule inhibitors. Either oral or intra-venous administration of the JNJ compound to mice bearing tumors demonstrated that the drug penetrates both the tumor microenvironment as well as the blood brain barrier. Subsequent experiments in a mouse intra-cranial tumor model demonstrated significant extension of survival in mice treated with this compound. These findings provide some hope for clinical development of an agent to treat CNS metastasis of Her2/neu/ErbB2 positive breast cancer. With a little magic, there may even be a success in treating or delaying the appearance of this unique clinical niche. But the ability to target ErbB2 receptor tyrosine kinases in the CNS would certainly not be limited to breast cancer. Clinical trials of gefitinib and erlotinib for newly diagnosed or recurrent glioblastoma have already been performed with somewhat disappointing results(Chakravarti et al., 2006; Haas-Kogan et al., 2005; Krishnan et al., 2006; Rich et al., 2004). However like many malignant cell types, gliomas express multiple ErbB RTK family members that each provide parallel proliferative and survival signals. Thus a pharmacologic agent that has enhanced activity against ErbB2 may improve responses in this disease as well.

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In addition, such an agent might open the door to the treatment of another compelling clinical need, medulloblastoma, the most frequent malignant pediatric brain tumor. At present treatment of medulloblastoma typically involves surgical excision followed by both radiation therapy to the brain and spine, and systemic chemotherapy. With this treatment overall prognosis for patients is excellent (with event free survival of ~85% at 5 years for patients with standard risk disease) but long-term toxicity can be severe; therefore, improvements certainly could be made in the therapeutic index. Recently a number of risk factors have been identified that portend a more aggressive clinical behavior in patients with medulloblastoma (Polkinghorn and Tarbell, 2007). Interestingly, ErbB2 is amplified and over-expressed in a sizeable sub-population of patients with medulloblastoma (Tong et al., 2004), and retrospective reviews have suggested that its expression is correlated with worse progression free and overall survival (Gilbertson et al., 1992; Gilbertson et al., 1995; Herms et al., 1997). In pre-clinical models, over-expression of ErbB2 in a medulloblastoma cell-line resulted in increased expression of genes associated with a metastatic phenotype and increased invasion (Hernan et al., 2003). Currently ErbB2 expression and its impact upon patient outcome is being prospectively analyzed on a series of multi-institutional trials and if its prognostic value is born out then an ErbB specific agent with good CNS penetration (such as JNJ-2871063) would satisfy the first 2 of the guidelines above. Therefore, it would not be unreasonable to evaluate if the addition of an ErbB2 inhibitor to conventional treatment may increase disease control and / or decrease the toxicity associated with the treatment of medulloblastoma.

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Near the turn of the 20th century the German scientist Paul Ehrlich envisioned a series of specific chemical agents or "magic bullets" as treatment for bacterial pathogens, a concept that guided his investigations of chemotherapeutic drugs later in his career. A scientist and clinician, Ehrlich was cognizant of the long time necessary to develop any clinical agent, remarking on his own efforts to develop a chemotherapeutic agent that "many year(s)… must elapse before a drug can be perfected which can even be tried on human beings". Now, more than 100 years later, we continue to struggle with both the issues that plagued Ehrlich as well as additional ones. Is the target present? can we hit it? and what is the clinical effect? are now accompanied by a fourth question:

How will tumor cells adapt to the targeted therapy?

Although multiple small molecule RTK inhibitors have been developed for groups or families of RTKs, this strategy for targeting tumor cells has had failures as well as successes, and just as Ehrlich foresaw, the true applicability of these agents will require much further pre-clinical and clinical evaluation.

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Figure Legend:

Figure 1: CNS penetration of monoclonal antibodies vs. small molecule inhibitors