



FINAL REPORT

Project Title: Crosstalk between EGFR and IGF1R mediated by polymorphisms in the EGFR promoter as a mechanism for resistance to IGF1R directed therapy in osteosarcoma

Project Number: SFA10-09

1. Date project was initiated: 7/2010
2. Period covered by this report: From 6/2010 To 7/2011
3. Publications, Abstracts, and Presentations:
 - a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry must include the author(s), article title, journal [book, editors(s), publisher, volume number, page number(s), and date.]

(1) Lay Press:

(2) Peer-Reviewed Scientific Journals:

Kolb EA, Kamara D, Zhang W, Lin J, Hingorani P, Baker L, Houghton P, Gorlick R. R1507, a fully human monoclonal antibody targeting IGF-1R, is effective alone and in combination with rapamycin in inhibiting growth of osteosarcoma xenografts. *Pediatr Blood Cancer*, 2010;55:67-75.

Sol-Church K, Kamara D, Stably D, Zhang WE, Lin J, Gorlick R, Kolb EA. A Single Nucleotide Polymorphism at -216 of the EGFR Promoter Predicts Increased EGFR Expression and Signaling in Response to IGF1R Inhibition. Manuscript to be submitted.

(3) Invited Articles:

Hingorani P, Kolb EA. Past, present and future of therapies for pediatric sarcomas. *Future Oncol*, 2010;6:605-618

(4) Abstracts:

- b. List presentations made during the last year (international, national, local societies, etc.). Use an asterisk (*) if presentation produced a manuscript.

*Kolb EA, Kamara D, Stabley D, Zhang W, Hingorani P, Gorlick R, Sol-Church K. A SNP at -216 of the EGFR predicts EGFR-IGF1R cross-talk and resistance to IGF1R inhibition in osteosarcoma. Oral Presentation, 2010 Annual Meeting Connective Tissue Oncology Society.

4. Provide a brief list of keywords: (limit to 20 words)

Sarcoma, IGF1R, EGFR, drug resistance, osteosarcoma, Ewing sarcoma.

5. Summarize the progress during the period of this report and its impact on your plans for the remainder of the project. Include a summary of the progress toward the achievement of the originally stated aims and list the significant results:

We were highly successful during the funding period by the Sarcoma Foundation of America. Specific Aim 1 was completed and the results published in the Sol Church manuscript currently in development as mentioned above. These data were also presented as an Oral Presentation at the 2010 Connective Tissue Oncology Meeting in Paris, France. Specific Aim 2 is ongoing. However, following completion of Specific Aim 1, it became clear that response to IGF1R-targeted therapies could be predicted by a single nucleotide polymorphism in the EGFR promoter. Instead of searching for signaling changes in peripheral blood mononuclear cells, we focused on identifying the frequency of this SNP in osteosarcoma tumors. Results are summarized below in the context of other published research.

Recently, several fully humanized monoclonal antibodies have shown promise in clinical trials. In a Phase 1 trial of R1507 in 37 patients with advanced solid tumors, 2 patients with Ewing's sarcoma had partial responses, and 13 patients had stable disease (1). While these results were not sufficient to warrant further clinical development of R1507, other IGF1R targeted therapies have shown equal promise in early phase clinical trials. As these agents move forward in clinical development, understanding markers and mechanisms of resistance will be imperative. Data resulting from this project demonstrate that EGFR expression may be influenced by signaling through IGF1R, and that this EGFR-IGF1R crosstalk may be predicted by EGFR promoter sequence. These are the first data to link inducible expression of EGFR in response to IGF1R signal inhibition to a polymorphism in the regulatory region of the EGFR promoter. The implication is that there is not only is there a somatic genetic signature for response to IGF1R inhibitors, but also a rationale for combining IGF1R and EGFR inhibition in a subset of patients.

The regulatory region of the EGFR promoter is GC rich with multiple transcriptional start sites, but no TATA box. In such promoters, Sp family of proteins often play a key role in activation (2, 3), as is the well described case of the EGFR promoter (4, 5). As the location one of four Sp1 recognition sites, it is reasonable to hypothesize that the polymorphism at -216 of the EGFR promoter is involved in regulation of EGFR expression. Liu et al. have reported that the G allele at -216 predicts lower constitutive expression of EGFR than the T allele. Data from this project confirm the same for constitutive EGFR expression in osteosarcoma. In the SaOS, OS31 and OS33 cell line (all G/G at EGFR-216) there is lower relative constitutive expression of EGFR by immunoblotting when compared to OS1 (TT at EGFR-216), OS2, OS9 and OS17 (G/T at EGFR-216). However, the opposite pattern is seen with inducible EGFR expression following inhibition of either IGF1R signaling or expression: the G allele predicts high inducible expression of EGFR in

response to IGF1R inhibition and, as a consequence, resistance to therapeutic inhibition of IGF1R. Reconciling the disparate influence of -216 sequences on constitutive and inducible promoter activity and EGFR expression will be difficult. As identified by Liu et al., there are numerous other function cis-acting elements in the EGFR promoter that may influence EGFR expression under different conditions. Key to understanding this mechanism will be the influence of IGF1R transcription factors regulating EGFR expression. These experiments are currently underway.

The significance of the SNP at -216 of the EGFR promoter relates both the fact that it is predictive of response to IGF1R inhibitors in OS and ESFT, and the fact that it offers a rationale for a combination of EGFR and IGF1R inhibitors in a subset of patients. Data from this project demonstrate therapeutic enhancement of the combination in the OS31 xenograft line, which is relatively resistant to both EGFR inhibitors and IGF1R inhibitors as single agents (6-10). The combination of IMC-A12, a fully humanized monoclonal antibody against IGF1R, and cetuximab, a human/mouse chimeric antibody against EGFR, in a randomized phase II trial yielded no significant results in patients with EGFR inhibitor resistant metastatic colorectal cancer (11). Clinical development of the combination in sarcomas may be limited by EGFR resistance. However, predictors of EGFR resistance in metastatic colorectal cancer such as *KRAS* mutations, lack of PTEN expression, *BRAF* or *PIK3CA* mutations are rarely reported in sarcomas (as reviewed in (12)). Nonetheless, despite the therapeutic enhancement in OS31 the tumor still grows, albeit slowly. These data confirm that EGFR may in part mediate resistance to therapeutic blockade of IGF1R, but also suggest that there are other signaling mechanisms maintaining tumor growth despite inhibition of IGF1R and EGFR.

The concept of EGFR-IGF1R crosstalk is not novel (reviewed in (13)). Barnes et al, demonstrated in head and neck cancer that IGF-induced signaling is enhanced by EGFR activation and/or EGFR/IGF1R scaffolding. In these cell lines, the combination of IGF1R and EGFR inhibitors was additive (14). Buck, et al. reported reciprocal activation of EGFR and IGF1R by inhibition of either receptor. Inhibition of MAPK activation by EGFR inhibitors releases negative feedback inhibition imposed on the IGF1R-insulin receptor substrate 1 (IRS-1) complex. In these studies, it is apparent that resistance to EGFR inhibition may be mediated by inducible signaling through IGF1R (15). Inducible signaling through EGFR in response to IGF1R inhibition was later reported in hepatocellular carcinoma cells (16). In cell lines resistant to a small molecule inhibitor of IGF-1R (BMS-536924), EGFR is constitutively overexpressed. Additive activity was observed for BMS-536924 used in combination with gefitinib, a small molecule inhibitor of EGFR, in the Rh26 rhabdomyosarcoma cell line (17). In the current report, EGFR is not constitutively overexpressed and would not be a useful biomarker for response. It is the SNP at -216 of the EGFR promoter that predicts inducible crosstalk between EGFR and IGF1R. The interaction between EGFR and IGF1R may occur through direct association of the receptors; altered expression and availability of ligands; or through common signaling partners such as G-protein coupled receptors and components of downstream signaling pathways (reviewed in (18)). It is apparent that resistance to EGFR inhibitors may be mediated by a release of feedback inhibition of IGF1R signaling; while resistance to IGF1R inhibitors may be mediated by EGFR expression and signaling. In the current report, we suggest that EGFR promoter sequence may predict inducible resistance to IGF1R inhibitors in 50% of patients. This is the first report that links IGF1R signaling with transcriptional regulation of EGFR. These data provide further rationale for combining inhibitors of EGFR with inhibitors of IGF1R, and identify patients likely to benefit most from the combination.

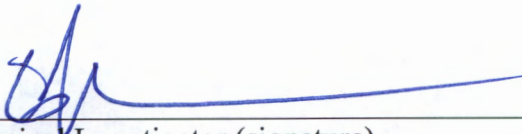
The data presented in this manuscript suggests a model where the absence of a signal through IGF1R induces cellular events capable of influencing mediators of EGFR transcription. IGF1R is capable of influencing transcription through multiple pathways, including Pi3K/Akt, JAK/STAT and Ras/MAPK (as reviewed in (19)). The efficiency of any or all of these IGF1R-regulated pathways to influence EGFR expression is determined by promoter sequence at -216 of the EGFR promoter. These findings may have implications across all tumor types treated with IGF1R inhibitors. The frequency of the G/G SNP at -216 is 39.7% in individuals of European descent and 93.4% in East Asians (20). In 50 OS patient samples evaluated in this report, the EGFR-216(G/G) genotype was found in 47% of the samples. In 11 xenograft models of pediatric sarcomas, this SNP at -216 of the EGFR promoter is capable of predicting resistance to 4 different inhibitors of IGF1R. Confirming the role of this SNP in transcriptional regulation in response to

IGF1R signal inhibition, and defining the mechanism by which IGF1R can exert influence on the EGFR promoter will be crucial as IGF1R-target therapies precede in clinical trials.

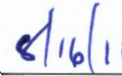
6. In layperson's terms, summarize the progress during the period of this report.
Explain any medical significance or implications of your results to date:

New therapies are needed in osteosarcoma to improve survival especially among patients with recurrent or metastatic disease. Insulin like growth factor-1 (IGF-I) is essential for growth in osteosarcoma. Several of the fully humanized monoclonal antibodies targeting the IGF-I receptor (IGF-IR) currently in clinical trials have clear evidence of anti-tumor activity in osteosarcoma tumor lines. However, some osteosarcoma lines remain resistant to IGF signal inhibition. Preliminary data obtained with funding from the Sarcoma Foundation of America demonstrate a previously unknown link between epidermal growth factor receptor (EGFR) expression and IGF-IR inhibition. Tumors resistant to IGF-IR inhibition induce expression of EGFR in response to treatment with R1507, a fully humanized anti-IGF-IR antibody. A polymorphism (gene sequence) in the EGFR gene predicts resistance to IGF1R targeted therapy and occurs in approximately 50% of osteosarcoma samples.

This project links response to IGF1R targeted therapies to a single nucleotide polymorphism in the EGFR gene and offers rationale for combined EGFR and IGF-IR inhibition in osteosarcoma. These data will guide the identification of rational therapeutic combinations with other targeted agents and identify markers of response. In essence, this project offers a easily identifiable marker for response to IGF1R targeted therapy. Validation of this marker in clinical trial will provide a patient-specific, personalized guide to appropriate therapy.



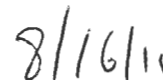
Principal Investigator (signature)



Date



Department Chair (signature)



Date

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