Istiratumab (MM-141), a bispecific antibody targeting IGF-1R and ErbB3, inhibits pro-survival signaling *in vitro* and potentiates the activity of standard of care chemotherapy *in vivo* in ovarian cancer models

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Abstract

Insulin-like growth factor receptor 1 (IGF-1R) signaling has been implicated in the pathogenesis of ovarian cancer (OvCa). However, clinical trials evaluating monospecific IGF-1R inhibitors have demonstrated limited clinical efficacy. Our data indicate that ErbB3, a member of the ErbB receptor tyrosine kinase family, can activate pro-survival AKT signaling in response to IGF-1R blockade and may represent a potential escape route in the development of resistance to therapy. Istiratumab (MM-141), an IGF-1R and ErbB3 directed bispecific antibody, inhibits ligand activation of these signaling pathways and degrades IGF-1R and ErbB3 receptor-containing complexes, leading to inhibition of downstream pro-survival signaling. Here we tested the activity of istiratumab, alone and in combination with chemotherapy, in *in vitro* and *in vivo* models of ovarian cancer.

Anti-proliferative activity of istiratumab monotherapy was evaluated in a panel of ovarian cancer cell lines *in vitro*. The effects of istiratumab and the ligands IGF-1 and heregulin (HRG) on IGF-1R- and ErbB3-mediated survival signaling were tested by ELISA and immunoblotting. Co-treatment assays with istiratumab and chemotherapy investigated mechanisms of synergy and additivity. Anti-tumor activity of istiratumab, alone and in combination with chemotherapy, was tested in an *in vivo* ovarian xenograft tumor model.

Our results indicated that istiratumab monotherapy inhibits ovarian cancer cell line proliferation *in vitro*. In addition, istiratumab blocked ligand-mediated resistance to chemotherapy. Co-treatment of istiratumab, ligands or chemotherapy indicated a strong correlation between drug activity and IGF-1R expression. Furthermore, co-treatment of chemotherapies and ligands potentiated AKT activation, which was inhibited by istiratumab. *In vivo* studies showed that istiratumab potentiates the activity of chemotherapy in ovarian xenograft tumor models.

Our findings demonstrate that co-inhibition of IGF-1R and ErbB3 signaling with istiratumab can potentiate standard of care chemotherapies in ovarian tumor models and warrant further investigation of istiratumab as a potential therapy for ovarian cancer patients.

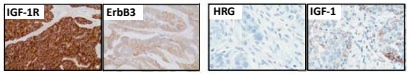
IGF-1R, ErbB3 and Their Ligands are Prevalent in High-Grade Serous Ovarian Cancer

- IGF-1R, ErbB3 or their ligands were identified in more than 95% of ovarian tumor samples tested, suggesting the pathway may be important in ovarian pathogenesis

Istiratumab biomarker prevalence in ovarian tumors (N=171)

IGF-1R	ErbB3	IGF-1	IGF-2	HRG
96%	88%	44%	47%	25%

Receptor expression (IHC) **Ligand expression (RNA-ISH)**

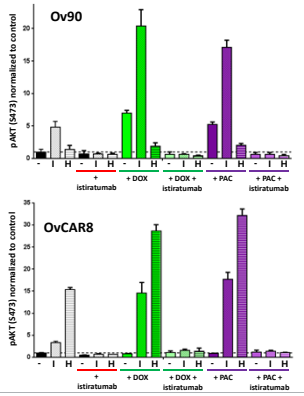


Receptor and ligand expression evaluated using immunohistochemistry (IHC) and RNA in situ hybridization (RNA-ISH), respectively

Tumor Treatment History
Treatment naïve: N=147
Post-chemotherapy treated: N=24

Cutpoints
IGF-1R, ErbB3: IHC 1+
IGF-1, IGF-2, HRG: ISH 1+ in tumor cells

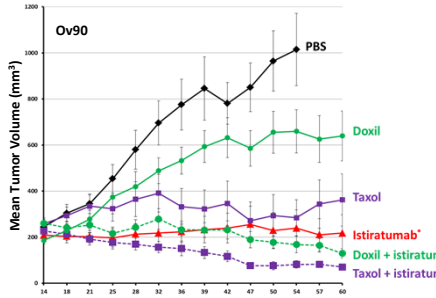
Istiratumab Inhibits AKT Activation Potentiated by Chemotherapy and Ligand Co-Treatment



- Ovarian cancer cell lines display differential sensitivity to exogenous IGF-1 and/or HRG
- Co-treatment with chemotherapy + ligand significantly increases AKT activation
- Istiratumab significantly inhibits chemotherapy + ligand-induced AKT activation

-, no ligand
H, HRG
I, IGF-1
DOX, doxorubicin
PAC, paclitaxel (Taxol)

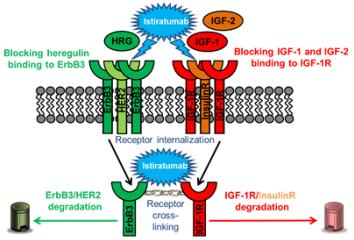
Istiratumab + Taxol or Doxil Inhibits *In Vivo* Tumor Growth



Doxil: 3 mpk, i.v., QW; Taxol: 20 mpk, i.p., QW
Istiratumab: 30mpk, i.p., Q3D
$p<0.0005 versus istiratumab; *$p<0.005$ versus Doxil; ᵗp<0.05 versus istiratumab

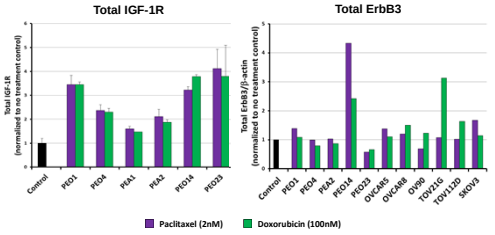
Istiratumab Targets Both IGF-1R and ErbB3

- Fully human, tetravalent, bi-specific antibody targeting IGF-1R and ErbB3
- Istiratumab is a homodimer containing two sets of identical polypeptide chains:
  - IgG antibody heavy and light chain targeting IGF-1R
  - scFv antibody fragment targeting ErbB3 at the C-terminus
- Blocks cognate ligand binding to IGF-1R and ErbB3, and triggers degradation of receptor complexes containing IGF-1R and ErbB3 and their heterodimers
- By inhibiting receptor activation and removal of receptors from the cell surface, istiratumab leads to significant decreases in pro-survival PI3K/AKT/mTOR signaling



Chemotherapy Treatment Increases Expression of IGF-1R and ErbB3 Receptors in OvCa Cell Lines

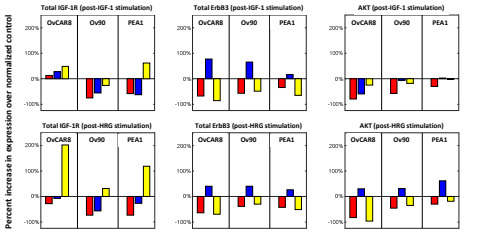
- IGF-1R and/or ErbB3 receptor expression increased 24 hours post-treatment with paclitaxel or doxorubicin in a subset of ovarian cancer cell lines
  - Changes in IGF-1R expression were measured by ELISA
  - Changes in ErbB3 expression were measured by western blotting



The ovarian cancer cell lines PEO1, PEO4, PEO14, PEO23, PEA1 and PEA2 were licensed from Cancer Research Technology, UK

Istiratumab Inhibits Redundant Receptor Upregulation and AKT Activation Post-DOX + Ligand Co-Treatment

- OvCa cell lines were treated with Dox (24 hr), ligand (15 min) followed by istiratumab or monospecific antibody (mAb) targeting IGF-1R or ErbB3 (15 min – 24 hr)
  - Treatment with DOX, ligand and a IGF-1R mAb leads to ↑ErbB3 expression; and treatment with DOX, ligand and a ErbB3 mAb leads to ↑IGF-1R expression
  - Supports the hypothesis that the dual blockade is needed to prevent redundant receptor activation of pro-survival AKT signaling



Summary

- Istiratumab was designed, using an integrated Systems Biology-based approach, to inhibit pro-survival signaling in cancer cells by co-blocking both IGF-1R and ErbB3 receptors
- IGF-1R, ErbB3 and/or their ligands can be identified in more than 95% of ovarian tumor samples
- Standard of care chemotherapeutics upregulate IGF-1R and ErbB3 receptor expression in panel of ovarian cancer cell lines
- Istiratumab inhibits AKT activation potentiated by chemotherapy + ligand co-treatment
- Istiratumab + Doxil and istiratumab + paclitaxel both induce ovarian tumor regression *in vivo* compared to single agent therapy
- These findings warrant further investigation of istiratumab as a potential therapy option for patients with ovarian cancer