

CR014-vcMMAE, a potent fully human monoclonal antibody-monomethylauristatin E-conjugated drug targeting ovarian and renal cell carcinoma



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Abstract

Ovarian and renal cell carcinomas are highly drug-refractory neoplasms representing significant unmet medical needs. To identify potentially novel therapeutic targets for these malignancies, we employed differential transcript profiling and identified a human gene, Tim1 (HAVCR1, Kim1), that is highly expressed in human ovarian as well as renal cell carcinoma tissues and cell lines. Our data confirm and extend previous findings that Tim1, a type I transmembrane protein, is a putative dedifferentiated kidney epithelial cell as well as renal cell carcinoma biomarker. We developed a high affinity fully human monoclonal antibody, designated CR014 to the Tim1 extracellular domain. CR014 showed specific binding to the surface of ovarian and renal carcinoma cell lines by flow cytometry and immunoblotting Tim1 protein from cell lysates. Detailed immunohistochemical analyses revealed significant Tim1 expression in the majority ovarian and renal cell carcinoma clinical samples examined, with a restricted normal tissue distribution. Therefore, Tim1 may represent a suitable target for monoclonal antibody-based therapy of ovarian and renal cell carcinoma.

In support of this approach, exploratory studies showed that CR014 inhibited both ovarian and renal cell carcinoma cell line growth when grown in the presence of a saporin-conjugated secondary antibody. Based upon these findings, a fully human monoclonal antibody-drug conjugate (ADC) was generated by covalently coupling CR014 to the potent cytotoxic agent monomethylauristatin E (MMAE) via the highly serum-stable, yet intracellular, protease-sensitive valine-citrulline (vc) peptide linker. This fully human antibody-conjugated drug, designated CR014-vcMMAE, retained binding to cell surface Tim1 and potently inhibited the growth of Tim1-positive cancer cell lines (IC50 range=26-176 pM), but had no effect on antigen-negative cell lines under these conditions of exposure. A control antibody conjugated with vcMMAE demonstrated neither binding to, nor growth inhibition of, Tim1-positive cell lines.

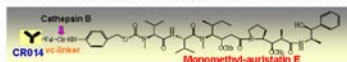
In xenograft mouse models of ovarian and renal cell carcinoma, CR014-vcMMAE induced significant and substantial dose-dependent anti-tumor effects. Complete regressions were noted in both the IGROV-1 ovarian and the Caki-1 renal cell carcinoma models at doses which showed no measurable toxicity. These data indicate that CR014-vcMMAE directed against Tim1 may be a highly potent and selective agent for the treatment of ovarian and renal cell carcinoma.

Renal Cell and Ovarian Carcinoma

- The American Cancer Society estimates that in 2006, 38,890 Americans will be diagnosed with renal carcinoma and 12,840 people will die from this disease. The incidence of people developing kidney cancer has been increasing at a rate of about 1.5% per year.
- An estimated 20,180 American women will be diagnosed with ovarian carcinoma in 2006 and during the year there will be an estimated 15,310 deaths. A woman's risk of having ovarian cancer during her lifetime is 1:58.
- Although surgery, combination chemotherapy, radiotherapy and biological agents may provide some survival benefit, more effective therapies are needed.
- Antibody-based therapy is an attractive approach for this unmet medical need.
- Genome-wide expression profiling and comprehensive bioinformatics analysis has identified an integral cell surface glycoprotein, Tim1, that is highly expressed on most renal and ovarian carcinoma cell lines and clinical samples.

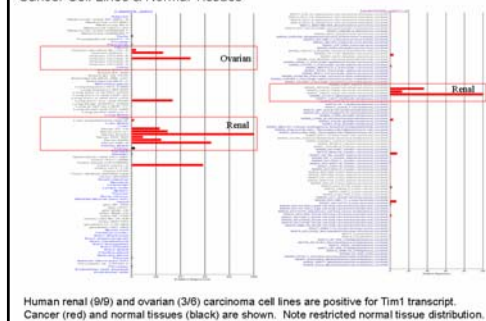
Tim1 and CR014

- Tim1 is a type I transmembrane protein containing Ig-like and mucin domains. Tim1 also contains a cytoplasmic tyrosine phosphorylation site.
- Tim1 is identical to HAVCR1/Kim-1 and may be regulated by tumor hypoxia. Tim1 is thought to play a role in CD4+ T-cell activation and atopy. CuraGen has recently shown that a soluble engineered form of Tim1 is immunosuppressive.
- Tim1 transcripts are strongly expressed in many of the human renal and ovarian carcinoma clinical samples and cell lines examined.
- A fully human monoclonal antibody (designated CR014) to the extracellular domain of Tim1 was generated by Xenomouse technology. $K_D=2.71 \times 10^{-8}$.
- CR014 was subsequently conjugated to monomethyl-auristatin E (MMAE), an inhibitor of tubulin polymerization, using a valine-citrulline (vc) peptide linker, and the resultant immunocjugate is designated CR014-vcMMAE (see figure below).



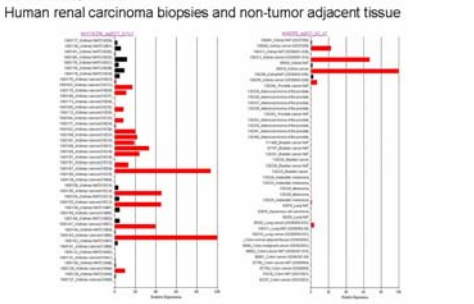
- Characterization of both unconjugated CR014 and its drug conjugate CR014-vcMMAE are presented.

Tim1 RTQ-PCR: Relative Transcript Expression



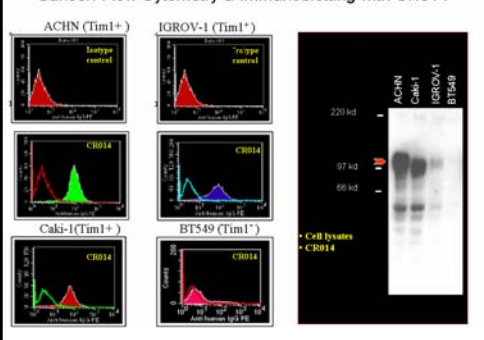
Human renal (9/9) and ovarian (3/6) carcinoma cell lines are positive for Tim1 transcript. Cancer (red) and normal tissues (black) are shown. Note restricted normal tissue distribution.

Tim1 RTQ-PCR: Human renal carcinoma biopsies and non-tumor adjacent tissue

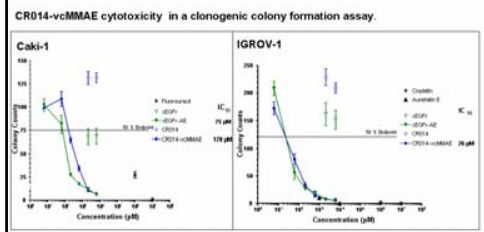


Human renal carcinoma biopsies (red) and non-tumor adjacent tissue (black) are shown. At least 11/28 samples demonstrate greater than 5-fold relative increase in mean Tim1 transcript.

Tim1 Expression in Renal (ACHN, Caki-1) & Ovarian (IGROV-1) Cancer: Flow Cytometry & Immunoblotting with CR014



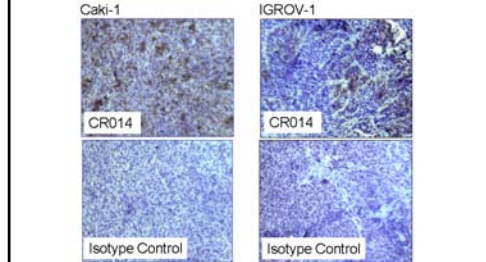
Cancer Cell Line Growth Inhibition with CR014-vcMMAE



Methods: Cells seeded at 2500 cells/well in 96-well plates were incubated with indicated reagents for 4 days, harvested and transferred to 6-well plates for the colony formation assay. Surviving stained colonies were counted.

Results: Unconjugated CR014 mAb did not show any anti-proliferative activity. CR014-vcMMAE potently inhibited the growth of Caki-1 (IC₅₀ = 170 pM) and IGROV-1 (IC₅₀ = 26 pM). CR014-vcMMAE had no effect on the Tim1-negative breast cancer cell line, BT549 at similar concentrations (data not shown).

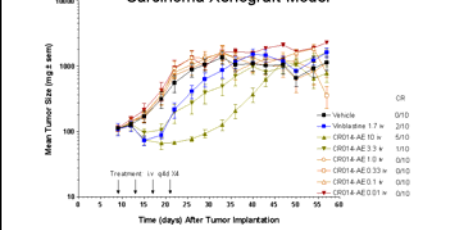
Immunohistochemical Detection of Tim1 in Caki-1 and IGROV-1 Xenografts by CR014



Methods: Athymic mouse xenograft samples were stained with biotin-conjugated CR014 mAb followed by streptavidin conjugated horseradish peroxidase staining and counterstained in hematoxylin and eosin.

Results: CR014 specifically stained the Tim1 antigen in renal cell and ovarian carcinoma xenografts.

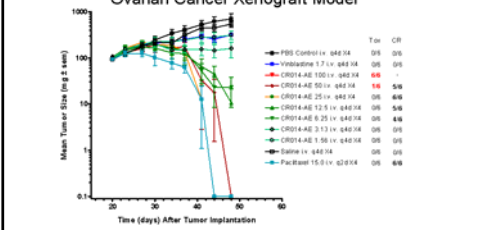
CR014-vcMMAE Anti-Tumor Effects in a Caki-1 Renal Carcinoma Xenograft Model



Methods: Athymic mice were implanted subcutaneously with Caki-1 tumor fragments. After tumors became established, test reagents were administered by iv injection (lateral tail vein). Tumor size (in mg) was measured by Vernier calipers twice weekly and calculated by the formula, (W³ x L)/2. Animals with tumors > 2g were removed from the study. For graphing purposes, test animals showing complete regressions were assigned a nominal tumor size of 0.10 mg before means were calculated.

Results: CR014-vcMMAE produced substantial anti-tumor effects in treated animals, and complete regressions (CR) occurred in a dose-dependent manner. CR014-vcMMAE treatment caused no observable effects on body weight in treated animals.

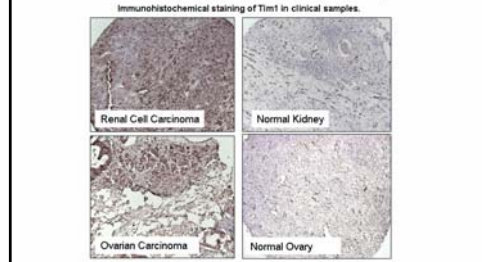
CR014-vcMMAE Anti-Tumor Effects in the IGROV-1 Ovarian Cancer Xenograft Model



Methods: Athymic mice were implanted subcutaneously with IGROV-1 tumor fragments. After tumors became established, test reagents were administered by iv injection (lateral tail vein). Tumor size (in mg) was measured by Vernier calipers twice weekly and calculated by the formula, (W³ x L)/2. Animals with tumors > 2g were removed from the study. For graphing purposes, test animals showing complete regressions were assigned a nominal tumor size of 0.10 mg before means were calculated.

Results: CR014-vcMMAE produced substantial anti-tumor effects in treated animals. Complete regressions (CR) occurred in a dose-dependent manner. No indication of tumor growth 150 days post-treatment. At high doses of 60-100 mg/kg treatment (i.e., cumulative doses of 200-400 mg/kg, toxicity was observed).

Immunohistochemical Analysis of Tim1 in Renal Clear Cell and Ovarian Carcinoma Clinical Samples



Methods: Clinical samples were stained with biotin-conjugated CR014 antibodies followed by streptavidin-conjugated horseradish peroxidase staining and counterstained with hematoxylin and eosin.

Results: CR014 specifically stained Tim1 in 74% (42/57) renal and 69% (11/16) ovarian carcinoma specimens. Normal tissue staining was limited to tissue lymphocytes, macrophages, isolated cells of ovary and isolated kidney proximal tubules (data not shown).

Summary

- Renal and ovarian carcinomas are incurable diseases. Current chemotherapeutic agents show low response rates (<20%).
- Transcription profiling and immunohistochemical staining demonstrated that Tim1 is highly expressed in renal as well as ovarian carcinoma and represents a promising target for an immunotherapy.
- Flow cytometric analysis demonstrated that Tim1 is expressed on the surface of renal and ovarian carcinoma cells.
- A high affinity monoclonal antibody, CR014, generated against Tim1 recognized cell-surface Tim1 protein and specifically immunoblotted Tim1.
- CR014 possessed potent anti-proliferative activity on antigen positive renal and ovarian carcinoma cell lines when conjugated to vcMMAE.
- CR014-vcMMAE administered to test animals bearing Caki-1 and IGROV-1 xenografts induced significant tumor regression without toxicity or weight loss.
- These data suggest that CR014-vcMMAE directed against Tim1 may be a highly potent and selective agent for the treatment of renal and ovarian carcinoma.