Evaluation of eribulin-induced alterations of the intact immune cell landscape in spleens and tumors from tumor-bearing immunocompetent mice

Abstract 5522

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Background

Eribulin, a synthetic analog of the marine sponge natural product halichondrin B,[1] is approved in the US for certain patients with advanced breast cancer or liposarcoma.[2] Its mechanisms of action include both cytotoxic, antimitotic effects as well as non-cytotoxic effects on tumor vasculature, phenotype and microenvironment, including reversal of epithelial-mesenchymal transition (EMT) and induction of cellular differentiation in various preclinical models.[3,4] Current literature suggests that EMT reversal should be associated with reduced immunosuppression,[5,6] and indeed a recent clinical study reports reduced levels of immunosuppressive PD-1, PD-L1, PD-L2, Foxp3+ regulatory T cells (Tregs) and increased CD8+ cytotoxic T cells in tumors of breast cancer patients who responded to eribulin therapy.[7] Some years ago, our group reported preliminary studies investigating eribulin's effects on the immune system in immunocompetent mice bearing syngeneic Lewis lung carcinoma (LLC) isografts.[8] Those results showed that eribulin treatment enhanced tumor-induced splenomegaly resulting from extramedullary hematopoiesis (EMH), and pointed to proliferation of splenic non-lymphoid, non-myeloid hematopoietic progenitors as the cause of the observed splenomegaly. Here we extend those early studies in both LLC tumors and another eribulin-responsive murine syngeneic tumor, Renca kidney cancer, by examining both tumor- and eribulin-induced effects on immune cells present in both spleens and tumors. Our results support the concept that eribulin has direct effects on the host immune system in both tumor-bearing and tumor-free mice.

Materials and Methods

Lewis lung carcinoma (LLC) and Renca kidney cancer murine syngeneic tumors were grown as isografts *via* cell inoculation in C57BL/6 and Balb/c mice, respectively (both fully immunocompetent), using 10 mice/group. Upon achieving mean group tumor volumes (TVs) of approximately 100 mm3, treatment with vehicle or 1.6 mg/kg eribulin was initiated using *iv* tail vein administration on a Q4Dx3 schedule. The dose of 1.6 mg/kg was chosen based on a previous dose/response pilot study using both models (not shown), and represented the highest tested dose that did not exceed the maximum tolerated dose (MTD) based on standard body weight considerations. Two and one experiments were done using LLC and Renca models, respectively. The first LLC study (termed LLC-1) included 2 tumor-free, untreated mice, while the second LLC study (LLC-2) and the Renca study both included 5 each tumor-free mice of appropriate strain for both vehicle- and eribulin-treated conditions. Four days following the last dose administration, animals were humanely euthanized, with spleens harvested for weighing and flow cytometry, and tumors harvested and preserved *via* formalin fixation and paraffin embedding (FFPE) for later immunohistochemistry (IHC) evaluation. Flow cytometry for the markers indicated in the figures was performed using standard procedures on a Becton-Dickinson LSRFortessa X-20 flow cytometer. High capacity IHC and analysis of immune infiltrates in FFPE-preserved tumor samples were performed at 40x magnification using a Leica Bond RX automatic IHC&ISH system with a Hamamatsu NanoZoomer-2.0 Digital Slide Imaging System. Statistics for group analyses were by one-way ANOVA using Sidak multiple comparison correction based on statistical hypothesis testing approach. Statistics for single point comparisons (e.g., *in vivo* time course) were by separate unpaired, 2-tailed t-tests for each time point. Statistics for paired x,y correlations were by simple linear regression, with 95% confidence bands shown in dotted lines, and probability of non-zero slope reported as a p value.

Eribulin inhibited LLC and Renca tumor growth

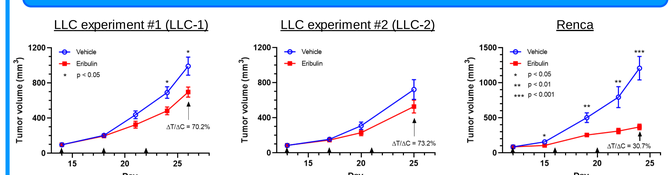

Figure 1. LLC and Renca tumor allografts were grown in C57BL/6 and Balb/c mice, respectively. Mice were treated on the days shown (arrows on x-axis) with vehicle or 1.6 mg/kg eribulin *iv* dosing on a Q4Dx3 schedule, with tumor volume measurements as shown. Not shown are non-tumor-bearing healthy controls, consisting of 2 untreated (LLC-1) or 5 each vehicle- or eribulin-treated mice (LLC-2, Renca) of the appropriate strains. Spleens and tumors were harvested 4 days following the last dose administration for the analyses shown in the subsequent figures. Results shown are means +/- SEM.

Eribulin induced and/or enhanced splenomegaly in both tumor-bearing and tumor-free LLC and Renca models

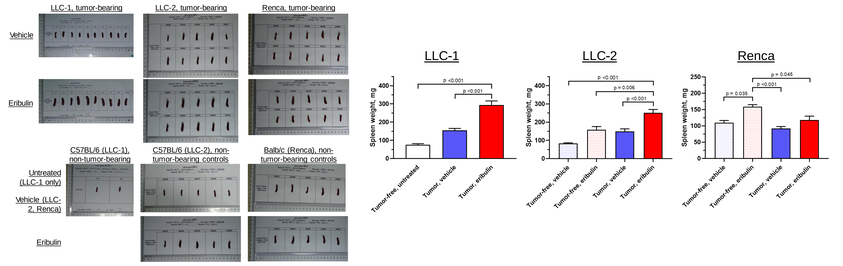

Figure 2. Spleens were harvested, photographed and weighed 4 days following the last dose administration for LLC-1, LLC-2 and Renca experiments (see Figure 1 legend). Group sizes were n = 10 for tumor-bearing groups. Two untreated, non-tumor-bearing C57BL/6 control mice were included in experiment LLC-1, while the LLC-2 and Renca experiments included 5 each vehicle- and eribulin-treated non-tumor-bearing C57BL/6 and Balb/c control mice, respectively. Histogram results represent means +/- SEM.

Larger spleens correlated with larger tumors in the LLC (C57BL/6) but not the Renca (Balb/c) model

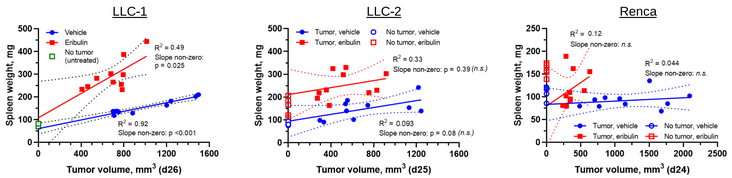

Figure 3. Correlations between tumor volumes and spleen size in individual mice.

Eribulin increased % and/or proliferation of splenic non-lymphoid cells in both tumor-bearing and tumor-free LLC and Renca models

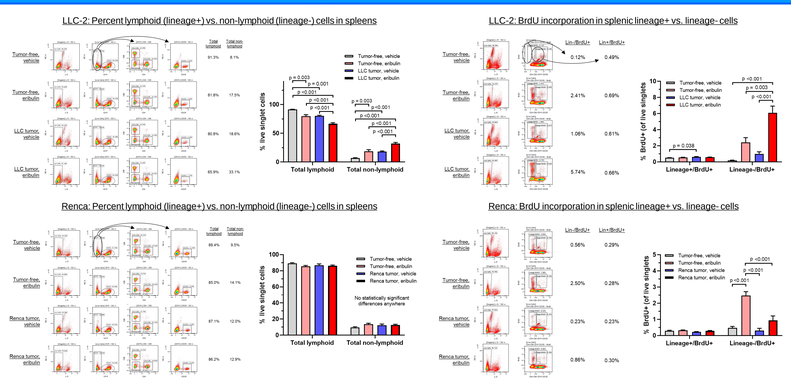

Figure 4. *Left:* Percent lymphoid *vs.* non-lymphoid splenic cells were determined by flow cytometric direct individual measurement of CD19+ (B cells), CD4+ and CD8+ (T cells) and CD335+ (NK cells) in LLC-2 and Renca experiments. *Right:* BrdU incorporation in lymphoid (lineage+) *vs.* non-lymphoid (lineage-) splenic cells was determined using a "dump channel" flow strategy employing a single fluorochrome (PerCP-Cy5.5) for x-CD19, x-CD4, x-CD8, x-CD335. For both left and right panels, bar charts show group means of 5 tumor-free and 10 tumor-bearing determinations for each condition, and representative flow data shown were selected to be as close to the group means as possible. Histogram results are means +/- SEM.

Eribulin increased both mature and immature splenic myeloid cells in both tumor-bearing and tumor-free LLC and Renca models

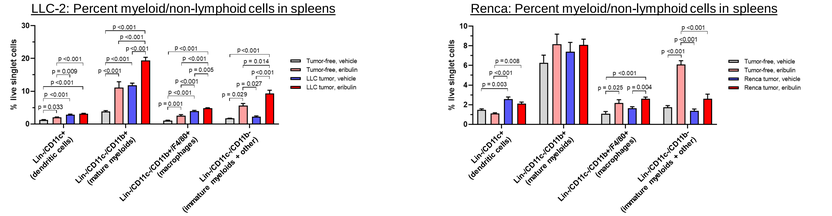

Figure 5. Percent individual splenic non-lymphoid (CD4-/CD8-/CD19-/CD335-; Lin-) cell types determined by stratified flow cytometry using markers shown. Data represent group means +/- SEM of 5 and 10 mice for tumor-free and tumor-bearing conditions, respectively.

Eribulin decreased tumor-associated macrophages in LLC (experiment #2 only), and increased CD4+ T cells and M1 macrophages in Renca model

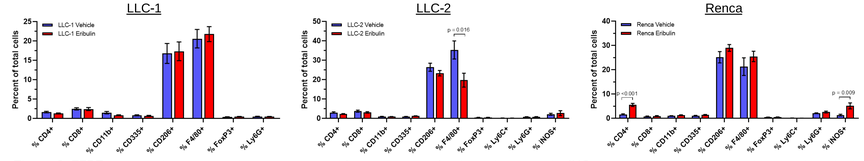

Figure 6. FFPE-preserved tumor samples were analyzed for expression of indicated markers by IHC using automated digital analysis as described in Materials and Methods. Results shown are means +/- SEM from 10 individual mice for each treatment condition, and represent evaluation of >400,000 and >100,000 total cells for each condition/marker for LLC and Renca models, respectively. CD206 and iNOS are markers for M2 and M1 macrophages, respectively.

Summary and conclusions

- Eribulin inhibits tumor growth in LLC and Renca murine allograft models, with Renca being the more sensitive model.
- Eribulin induced and/or enhanced splenomegaly in both tumor-bearing and tumor-free LLC and Renca models.
- Larger spleens correlated with larger tumors in the LLC (C57BL/6) but not the Renca (Balb/c) model.
- Eribulin increased percentages and/or proliferation of splenic non-lymphoid cells in both tumor-bearing *and* tumor-free LLC and Renca models
- Eribulin increased both mature and immature splenic myeloid cells in both tumor-bearing and tumor-free LLC and Renca models, including dendritic cells, mature myeloids, macrophages and immature myeloids/other cell types.
- Eribulin decreased tumor-associated macrophages in LLC (experiment #2 only), and increased CD4+ T cells and M1 macrophages (iNOS+) in Renca
- Overall, results in both LLC and Renca syngeneic tumor models are consistent with eribulin exerting direct effects on the host immune system, both in the spleen and in the tumors themselves. However, considering the magnitude and widespread nature of eribulin's effects in the spleen, its effects in tumor seem disproportionately low. One potential explanation for this is that such tumors, while syngeneic, are still artificially derived from subcutaneous injection of tumor cells, such that tumor 3D architecture and immune infiltrates are poorly representative of spontaneously derived tumors. Further studies in spontaneously developing murine mammary tumor models are contemplated to explore this further.

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Disclosures and acknowledgements

HW, KZ and XT are full-time employees of Crown Bioscience, where all of the studies presented on this poster were performed under paid contract with Eisai Inc. DA and MW-J are former employees of Eisai Inc., with their current affiliations as listed in the title line. BAL is a full-time employee of Eisai Inc., which manufactures and markets eribulin mesylate (as Halaven®) according to FDA-approved indications in the USA.