

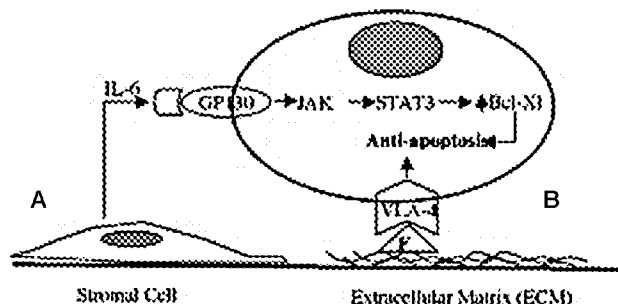
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INVITED

Environmental mediated drug resistance (EMDR) in hematologic malignancies

W.S. Dalton. H. Lee Moffitt Cancer Center, University of South Florida, Tampa, USA

Classically, studies of drug resistance in cancer have focused on the molecular biology of single cancer cells. These types of studies have provided important information regarding acquired drug resistance mechanisms, including mechanisms that reduce intracellular drug accumulation, alter or repair drug-induced damage, and reduce drug-induced apoptosis. Acquired drug-resistant models have been critical in elucidating intrinsic drug-resistant mechanisms; however, these models do not consider resistance mechanisms elicited by extrinsic influences such as the tumor microenvironment. We propose that the tumor microenvironment provides a sanctuary for subpopulations of tumor cells to evade or circumvent drug-induced death and that this represents a form of *de novo* drug resistance. Furthermore, this extrinsic form of *de novo* drug resistance contributes to minimal residual disease (MRD) resulting in the emergence of acquired drug resistance. We have found that elements of the bone marrow microenvironment, including extracellular matrices and stromal elements, protect hematologic malignant cells from drug-induced cell death. We propose that environmentally mediated drug resistance (EMDR) protects tumor cells from stress and cell death by two mechanisms: (A) a paracrine mechanism due to soluble cytokine factors produced as a result of the tumor cell:environment interaction, and (A) a physical contact mechanism we have termed "cell adhesion mediated drug resistance (CAM-DR)".



Two different forms of tumor-microenvironment interactions influence drug response in cancer.

Our initial studies comparing genotypic and phenotypic profiles of drug resistance indicate that EMDR is less complex compared to acquired drug resistance. Focusing on EMDR may reduce MRD and prevent the emergence of acquired drug resistance in hematologic malignancies. Two different forms of tumor cell-environmental interaction may explain how some tumor cells survive initial drug exposure and eventually express classical mechanisms of drug resistance. The first form involves soluble mediators, such as interleukins, that are secreted by non-tumor, stromal cells. Interleukin-6 (IL-6) is a classical example of how a soluble mediator secreted by the tumor microenvironment is capable of enhancing tumor cell survival and perhaps blocking apoptosis. The second form of tumor cell-environment interaction requires direct cell contact and has been given the term cell adhesion mediated drug resistance. In this case, binding extracellular matrix ligands, and bone marrow stromal elements in the tumor microenvironment may activate cell adhesion molecules, such as the integrins, and these interactions result in the activation of signal transduction pathways that block drug-induced apoptosis. Our work, as well as the work of others, have shown that the signaling pathways involved in EMDR are cell lineage, as well as, environmentally dependent. Interrupting the tumor cell-environment interactions or the associated signal transduction pathways may represent a new approach for the treatment of cancer by preventing MRD and the emergence of acquired drug resistance.

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Poster Sessions**Anthracyclines**

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POSTER

Efficacy of nemorubicin (MMDX) administered with iodinated oil via hepatic artery (IHA) to patients with unresectable primary hepatocellular carcinoma (HCC): phase II trial

Y. Sun¹, J. Yang², P. Luo³, Y. Zhang⁴, Y. Yan⁵, L. Sun⁵, M.A. Pacciarini⁶, O. Valota⁶, C. Geroni⁷. ¹Cancer Hospital, Chinese Academy of Medical Sciences, Oncology, Beijing, China; ²First Affiliated Hospital, Sun Yat-Sen University of Medical Science, Oncology, Guangzhou, China; ³Guangdong Provincial Peoples Hospital, Oncology, Guangzhou, China; ⁴Cancer Hospital, Zhongshan University, Oncology, Zhongshan, China; ⁵Pfizer, Oncology, Beijing, China; ⁶Nerviano Medical Science, Clinical Oncology, Nerviano, Italy; ⁷Nerviano Medical Science, Biology, Nerviano, Italy

MMDX is a doxorubicin (DX) derivative with different mode of action, superior therapeutic activity including activity on multidrug resistance tumor models and improved tolerability (mainly reduced cardiotoxicity relative to DX). The past clinical experience by IV route evidenced a special affinity of MMDX for liver lesions, whose regression in pts with colorectal, renal, breast cancer were reported in Phase (Ph) I-II studies (Vasey, *Cancer Res*, 1995; de Takats, *Proc ECCO* 9, 1997). The specific antitumor activity may be due to the formation of highly cytotoxic metabolite(s) by liver enzymes. These findings prompted to test the efficacy of MMDX by IHA in HCC. Ph I studies were conducted in EU and in China. The encouraging efficacy and safety results (Sun, *ASCO*, 2003) were supportive of continuing the development in HCC.

A registrative ph II/III randomized study was started in China in 2002, with MMDX administered by IHA in iodinated oil at 600 mcg/m² every 6 weeks to chemotherapy-naïve pts with unresectable HCC.

27 pts [25 male and 2 female (mean age 51.5, range 35–68 years); ECOG-PS 0 (21 pts) or 1 (6 pts); AJCC stage II (8 pts) and IIIA (19 pts); median number of cycles 2 (range 1–5)] were treated in the first stage of the Ph II portion of the study. 24 pts are currently evaluable for efficacy.

Partial remissions (PRs), evaluated by the WHO criteria occurred in 5/24 pts (4 confirmed) (RR = 20.8%; 95% c.i.: 7.1–42.2%).

Mild/moderate reversible neutropenia was reported in a small number of pts. Thrombocytopenia (Gr 3) occurred in 15% of pts (some pts were already thrombocytopenic at baseline). A mild/moderate SGOT/SGPT reversible increase occurred in 48% and 61% of cycles, respectively (max Gr 3 increase in 16% and 9.4% of cycles for SGOT and SGPT, respectively). No Gr 4 increase and no trend to cumulative liver toxicity with repeated administrations (neither in severity nor in frequency) were reported. Nausea and vomiting were also observed (Gr 3 vomiting in 11% of pts), being however mild/moderate in severity and resolving spontaneously in most cases without requiring antiemetic treatments. All the episodes recovered within 1 week. No Gr 4 gastrointestinal toxicity was observed.

Other mild/moderate toxic manifestations were pyrexia, anorexia, and fatigue. No toxic deaths occurred during the trial.

In conclusion, MMDX shows good activity against HCC and is well tolerated, the majority of the events being of severity Gr 1 or 2 and reversible in all cases. Altogether, the objective responses observed in Ph I-II studies by IHA, indicate an overall RR of 24.5% (13 PRs/53 HCC pts; 95% c.i.; 13.7–38.3%), confirming a significant activity of MMDX in this disease. A manageable safety profile coupled with a wide therapeutic index (objective responses obtained also at 200 mcg/m²) characterizes MMDX as a suitable compound for the IHA management of liver cancer.

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POSTER

In vitro and in vivo antitumor activity of nemorubicin metabolites

C. Geroni¹, R. Battaglia², M. Floreani³, D. Ballinari¹, A. Marsiglio¹, L. Quintieri³. ¹Nerviano Medical Science S.R.L., Biology Dept, Nerviano, Italy; ²Nerviano Medical Science, Global Drug Metabolism Dept, Nerviano, Italy; ³University of Padua, Pharmacology and Anesthesiology Dept, Padova, Italy

Nemorubicin (MMDX) is a doxorubicin (DX) derivative currently in phase II/III clinical trials for the treatment of hepatocellular carcinoma. As for other anthracyclines, the major metabolic pathway for MMDX, is the stereoselective reduction of the side chain C₁₃-carbonyl group, yielding the corresponding alcohol derivative, MMDXol. In terms of plasma AUC the MMDXol/MMDX ratio was about 0.1 in the rat, 3 in the dog, 0.7–1 in the monkey and 0.1 in humans. Moreover, MMDX undergoes hepatic oxidation by cytochrome P4503A4 to a more cytotoxic metabolite, PNU-159682, which has different DNA-interacting properties than MMDX.

In order to further characterize the pharmacological profile of MMDX, the two metabolites, MMDXol and PNU-159682 were tested in vitro toward a panel of drug-resistant cell lines and in vivo on murine and human tumor models.

Exponentially growing tumor cells resistant to DX (L1210/DX and LoVo/DX) or topoisomerase (topo) II inhibitors (CEM/VM-1) or topo I inhibitors (L1210/CPT and L1210/9-AC) and their sensitive sublines, were treated in vitro with the two metabolites. Efficacy was expressed as ratio between the IC₅₀ on resistant and sensitive cells (resistance index; RI). The in vivo antitumor efficacy and the tolerability of MMDX and its metabolites were tested on murine leukemias (L1210, P388 and P388/DX) or on human mammary carcinoma xenografts (MX-1).

MMDXol was 3–10 times less cytotoxic and PNU-159682 was 1300–4000 times more cytotoxic than MMDX in vitro. On DX resistant cells, MMDX, MMDXol and PNU-159682 were active, with RI of 2.6, 4 and 4, respectively, on L1210/DX and 1, 4 and 1, respectively, on LoVo/DX cells. Unlike other anthracyclines, MMDX does not interfere with DNA topo II functions and is effective on CEM/VM-1 cells. Both the tested metabolites overcame, as the parent drug, topo II-mediated resistance. The behavior of these compounds on topo I resistant cells was very interesting. MMDX (RI 148 and 80, respectively) and MMDXol (RI 12 and 7.5, respectively) were not effective while PNU-159682 shows activity with RI of 1 on both cell lines. In vivo, MMDXol showed activity comparable to that of MMDX on both P388 and P388/DX leukemias (ILS% 75 and 72, respectively) but at doses 14 times higher. PNU-159682 was as active as MMDX on L1210 murine leukemia but at doses 10 times lower. MMDX, MMDXol and PNU-159682 show a comparable good efficacy on MX-1 (99% tumor growth inhibition and cured mice).

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POSTER

Assessment of the therapeutic activity and dose optimization for the anthracycline RTA 744 (WP744) using a xenograph model

N. Thapar¹, C. Meyer², W. Huff², K. Culotta¹, I. Fokt³, W. Priebe³, T. Madden^{1,3}, M. Johansen^{1,3}. ¹University of Texas M.D. Anderson Cancer Center, Pharmaceutical Development Center, Houston, TX, USA; ²Reata Discovery, Inc, Dallas, TX, USA; ³University of Texas M.D. Anderson Cancer Center, Experimental Therapeutics, Houston, TX, USA

RTA 744, a 4'-O benzyl-modified anthracycline possessing superior activity against most tumor types when compared to doxorubicin, is now undergoing preclinical development. This compound differs from currently available anthracyclines by its ability to circumvent MDR1 and MRP1-mediated cellular efflux and enhanced activity in cells overexpressing Bcl-2 and its homolog Bcl-X_L. We previously described its pharmacology and toxicology in vivo and are now optimizing dose and scheduling using a human breast tumor xenograph model. Nude (nu/nu) mice (F, ~20 g) were subcutaneously implanted with MDA-MB-231 tumor cells and treatment was initiated when tumors reached 60–75 mg, approximately 10–15 days following implantation. Treatment groups (8 mice/group) were administered IP doses ranging from 5–25 mg/kg/dose using either a twice weekly (2XW)

or a daily × 5 (QDX5) dosage regimen. During treatment courses animals were monitored for body mass changes, tumor growth, and toxicity. Tumor measurements were obtained twice weekly and converted to tumor weight using a standard formula. Upon termination, mice were weighed, sacrificed and the tumors harvested. Mean tumor weight per group was used for comparison between cohorts with tumor growth inhibition (TGI) calculated for each group.

In groups receiving 5 and 6 mg/kg on a QDX5 schedule mean weight loss was 8.3% and 10.4%, respectively, at the end of the treatment course. Mean TGI was 49% in the 5 mg/kg QDX5 group and 63% in the 6 mg/kg QDX5 group. The groups receiving 15 and 25 mg/kg 2XW experienced weight loss of 15% and 30% and TGI of 61% and 71% respectively. At an equivalent cumulative weekly dose, increased TGI and less toxicity (evidenced by weight loss) was observed in the QDX5 versus the 2XW schedule. To refine the RTA 744 MTD, studies were conducted in non-tumor bearing mice administered doses of 7, 8, and 9 mg/kg IP QDX5 on a repeating schedule. Increased tolerability was observed with this dose schedule; animals in the 7 mg/kg group showed an 11.2% weight loss by day 7 which was almost totally reversed by the start of course 2. Interestingly, weight recovery began immediately following the second course in the 7 mg/kg group, while weight continued to decline for an additional 9 days in the 8 mg/kg group.

These data indicate that RTA 744 is active against human breast cancer and can be optimally administered using daily dosing schedules incorporating brief rest intervals. Future studies will examine more protracted dosage regimens, utilizing brief rest periods, to further optimize efficacy and minimize toxicity.

DNA repair

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POSTER

Poly(ADP-ribose) polymerase inhibitor, ABT-472 enhances antitumor activity of doxorubicin in human xenograft models and protects against drug-induced cardiac toxicity

I. Joseph¹, D. Ferguson¹, J. Palma¹, M. Refici¹, L. Godzicki¹, L. Rodriguez¹, D. Frost¹, S. Rosenberg¹, R. Grandel², W. Wernet².

¹Abbott Laboratories, Cancer Therapeutics, Abbott Park, USA; ²Abbott GmbH & Co. KG, Ludwigshafen, Germany

Poly(ADP-ribose) polymerase (PARP) is an essential DNA-damage sensing enzyme that facilitates DNA repair. Consequently, PARP may allow cancer cells to recover from chemotherapy-induced DNA damage. Inhibition of PARP (pharmacological or knockout studies) can enhance the toxicity of DNA-damaging agents. Therefore, the inhibition of PARP *in vivo* may increase the maximum therapeutic benefit of DNA-damaging chemotherapeutic agents. ABT-472 is a potent PARP inhibitor of both PARP-1 and PARP-2 enzymes (K_i, 2–2.5 nM and cellular IC₅₀, 20 nM) with a good PK profile. The ability of ABT-472 to enhance anti-tumor efficacy of doxorubicin was tested both *in vitro* and *in vivo* against the established HT-1376 human bladder carcinoma and A-253 head and neck xenografts grown in *scid* mice. *In vitro* studies demonstrated that ABT-472 increases doxorubicin sensitivity (2–3-fold) by increasing DNA strand breaks and enhancing cell death. HT-1376 tumor-bearing *scid* mice were treated with the maximally tolerated dose (MTD) of doxorubicin (0.67 mg/kg/day, i.p., for 3 cycles), ABT-472 (20.6 mg/kg/day, i.p., OMP) or the combination. The %T/C ratios on day 24 were 64, 101 and 48 and the %ILS values were 0, 0 and 64 for doxorubicin, ABT-472 and doxorubicin/ABT-472-treated groups, respectively. Efficacy of ABT-472 in combination with doxorubicin at MTD was also tested in A-253 tumor-bearing *scid* mice. The %T/C ratios on day 30 were 73, 77 and 35 and %ILS values were 21, 0 and 50 for doxorubicin (0.67 mg/kg/day, i.p., for 3 cycles), ABT-472 (10.3 mg/kg/day, i.p., OMP) and the combination group, respectively. Western blot analysis also demonstrated that ABT-472 inhibited doxorubicin-induced PAR accumulation in tumors *in vivo*. These studies demonstrate that ABT-472 significantly enhanced efficacy of doxorubicin in these xenograft models shown to be resistant to the drug at its MTD. ABT-472 alone did not show single agent anti-tumor activity. The combination of doxorubicin and ABT-472 was well tolerated. One of the major toxicities of the anthracycline class of drugs is cardiac toxicity (cardiomyopathy resulting from free radical-induced damage). Interestingly, ABT-472 protects against doxorubicin-induced acute cardiac damage indicated by survival of mice and inhibition of serum levels of troponin I and creatine kinase, markers for cardiac damage. Collectively, these studies demonstrate that ABT-472 significantly enhances the anti-tumor efficacy of doxorubicin in pre-clinical xenograft models by inhibiting doxorubicin-induced PAR accumulation. Moreover, not only is ABT-472 well tolerated in combination with doxorubicin, but ABT-472 also protects against doxorubicin-induced acute cardiac toxicity.