10 and 30 mg/kg doses. For pERK assessments in blood, BalbC mice were dosed at 1, 10 and 30 mg/kg and blood samples were taken at the same time points for PK and PD measurements. pERK was measured via flow cytometry in CD3+ lymphocytes after whole blood ex-vivo stimulation with phorbol myristate acetate. We applied a linear compartmental PK framework to describe plasma PK. The time course of tumor PD in-vivo was described by an indirect response model. A sigmoidal E_{max} model was used to describe the dose response relationship of blood PD. Fitted PK−PD models were then used to simulate the PD time course in plasma and tumor at efficacious doses in the A375 model. The models were further extended to simulate human PK−PD profiles.
Results: Simulations of blood and tumor PD profiles upon repeat dosing suggest that continuous and substantial inhibition of both tumor and blood PD is associated with drug response. The EC50s of tumor and blood PD were in broad agreement indicating biological relevance of measuring pERK inhibition in the blood. Simulations of the PD profile demonstrated that the trough PD response has better dynamic range than peak PD response, suggesting sampling strategies of blood PD should focus on trough levels.
Conclusions: A continuous and substantial inhibition of blood p-ERK level is expected to associate with TAK-733 response. Sampling time for PD response at trough levels offers an advantage to peak levels because of higher dynamic range.

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Comparative tissue distribution of the HDAC inhibitor JNJ-26481585

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Background: The histone deacetylase inhibitor JNJ-26481585 has been shown in preclinical testing to have significant efficacy against a number of solid tumour xenografts and improved potency when compared to other HDAC inhibitors such as Vorinostat (Arts et al, Clin Can Res 15, 6841, 2009). We hypothesised that this might be due to a combination of intrinsic potency against target HDACs and also improved tissue distribution. In this study we determined the comparative tissue distribution of JNJ-26481585 and compared it to that of other hydroxamic acid HDAC inhibitors.
Material and Methods: Male nude mice were dosed once per day by the oral route for up to 7 days with 40 mg/kg of Vorinostat, Panobinostat and JNJ-26481585. Dosed animals were sacrificed at 0, 0.5, 1, 2, 4, 7 and 24 hours (3 animals per timepoint), either for single or repeat dose, and plasma and tissues were prepared for compound analysis by LC-MS/MS. Tissues sampled included bone marrow, brain, heart, kidney, large intestine, liver, lung, muscle, prostate, skin and fat. In addition, tissue samples from skin, liver, lung and bone marrow were selected for immunohistological examination for markers of HDAC inhibition.
Results: Comparative exposures (AUC) showed highest levels of all compounds in the large intestine. After this, exposures were highest in the kidney, lung, prostate skin and heart. In all cases JNJ-26481585 showed superior tissue distribution to that of Vorinostat and Panobinostat, reaching levels up to 6 times those of Vorinostat in lung and up to 3 times in prostate, skin and kidney. JNJ26481585 showed better tissue penetration than Panobinostat particularly in brain, liver, muscle and skin. No tissue accumulation was noted after multiple dosing. Tissue levels of JNJ-26481585 exceeded those of plasma levels, with T/P ratios being in excess of 100 in large intestine, kidney and lung and over 50 in heart, prostate and skin.
Preliminary analysis of pharmacodynamic changes in tissues in response to the HDAC inhibitors showed a significant increase in histone acetylation concurrent with a significant reduction in the Ki67 marker of proliferation. Further comparative analysis will be presented.
Conclusions: JNJ-26481585 shows excellent tissue penetration in nude mice, superior to that of Vorinostat and Panobinostat. This property may make JNJ-26481585 an attractive candidate for clinical trials in solid tumours.

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Identification of HSP105 as a novel non-Hodgkin lymphoma restricted antigen

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Background: We reported that vaccination of relapsed indolent non-Hodgkin lymphoma (NHL) patients using dendritic cells loaded with killed autologous tumor achieved clinical benefits associated with humoral immunity. To identify novel NHL-restricted antigens (ags), we exploited the antibody (Ab) repertoire of responder patients (R) compared to that of non-R (NR), using both pre- and post-vaccine serum samples.
Methods: Purified pre- and post-vaccine Abs from R and NR were biotin-conjugated and tested by immunohistochemistry (IHC), flow cytometry (FC) and western blot (WB) both on autologous and allogeneic NHL specimens and cell lines. Ag discovery was performed applying a modified serological proteomic-based approach (SERPA) followed Mass Spectrometry (MS) analysis. MS-identified cancer-related proteins were further investigated for their role in lymphomagenesis.
Results: By IHC and FC, we found that post-vaccine Abs from R reacted not only on autologous but also on allogenic NHL biopses and cell lines at significantly higher levels than matched pre-vaccine R samples or NR pre- and post-vaccine Abs, respectively. Furthermore, Abs from post-vaccine R serum significantly impaired NHL cell line growth when added for 72 hours in culture as compared to Abs from normal human serum ($p = 0.001$). Towards the identification of novel potential targets for NHL, WB analyses of the follicular lymphoma (FL) cell line DOHH2, tested either as total cell lysate or acidic protein fractions, revealed one differential band migrating at about 100 kDa only when post-vaccine samples from R was used. MS analysis identified the heat shock protein (HSP) 105 as possible ag candidate. By FC, we observed that HSP105 was expressed both on the tumor cell surface and in the cytoplasm of a panel of B-NHL cell lines and, at lower levels, in normal B cells. On the other hand, no reactivity was found following FC analysis of normal T cells or T- lymphoma cell lines. In addition, by IHC on 50 lymphoma specimens, we determined that HSP105 expression levels increased at the increasing of tumor aggressiveness. Accordingly, in vitro blocking assays using a commercial anti-HSP105 rabbit serum revealed a higher anti-tumor activity directed to Burkitt's lymphoma than diffuse large B cell lymphoma or FL cell lines, respectively.
Conclusions: Our preliminary results suggest that HSP105 may represent a novel B-NHL-restricted ag that could be exploited as potential immunotherapeutic target. In vivo studies are ongoing to corroborate our working hypothesis to target HSP105 for the treatment of B-cell lymphoma.

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Baseline circulating tumor cell (CTC) counts enhance the performance of the Royal Marsden Hospital (RMH) Prognostic Score and improve patient selection for phase 1 clinical trials

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Background: CTCs are prevalent in many patients with advanced cancer with higher CTC counts portending a worse prognosis. The use of the RMH Prognostic Score for patient selection for phase 1 clinical trials has been previously validated in prospective analyses. (Arkenau et al, JCO 2009). We evaluated the incorporation of baseline CTC counts to further improve the utility of this prognostic score and enhance patient selection in phase I trials at the RMH.
Methods: We performed a retrospective analysis on the patients who had CTC enumeration as part of their phase 1 trial between January 2006 and December 2009. Blood samples were collected at baseline, during and post therapy for CTC counts and analysed using the CellSearch system (Veridex). Patient characteristics and baseline CTC counts were correlated with the RMH Phase 1 Prognostic Score, which is based on 3 objective markers (albumin <35 g/dL, lactate dehydrogenase [LDH] > upper limit of normal [ULN], and >2 sites of metastases).
Results: Data from 128 patients, male:female ratio (1.1:1), median age 60.5 years (range, 17.5–79.1 years) were collected. The most frequent tumor sites were genitourinary ($n = 31$), gastrointestinal ($n = 30$) and breast ($n = 18$). Median CTC count was 1 (range 0–134). Multivariate analysis indicated that both higher baseline CTC counts and RMH Prognostic Score were independent prognostic factors (HR 1.014, $p = 0.006$). The addition of baseline CTC count enhanced the performance of the RMH Prognostic Score and classified patients eligible to participate in Phase 1 clinical