es could solely be responsible for the anti-tumour activity, and although we did not examine specific immune responses against minor histocompatibility antigens, these would undoubtedly be present since the majority of these patients developed clinically evident GVHD. However, the finding that we are able to demonstrate in vivo donor specific immune responses against TAAs such as Id, that are capable of killing primary tumor cells, provides the rational for the development of clinical programs aimed at maximizing specific immune responses. We are currently performing pre-clinical studies aimed at generating TAA specific T cells for subsequent infusion to patients as an alternative to non-specific donor lymphocyte infusions.

UNDERSTANDING, DIAGNOSING, AND TARGETING BCL-2 ADDICTION

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It has been hypothesized, and is generally accepted, that a block in apoptosis is required for oncogenesis. Cancer cell behaviors, including metastasis, genomic instability, and oncogene activation generate death signals which must be blocked for cancer cells to survive. One potential mechanism cancer cells may exploit is expression of anti-apoptotic proteins of the BCL-2 family, including BCL-2, MCL-1, BCL-XL, BCL-w, and BFL-1. These proteins bind and sequester death signals carried by pro-death members of the BCL-2 family, preventing the initiation of programmed cell death. Cancer cells in which anti-apoptotic proteins tonically bind death signals may be *addicted* to anti-apoptotic function. We have developed a strategy, which we call *BH3 profiling* for diagnosing cells with addiction to BCL-2, MCL-1, BCL-XL, BCL-w, or BFL-1. We demonstrate that the biochemical basis for such addiction in lymphoid cancers appears to be sequestration of pro-death molecules like BIM by the anti-death molecules. Moreover, we show that cells identified as *addicted* to BCL-2 are uniquely and exquisitely sensitive to antagonism of BCL-2 function by the small molecule BCL-2 antagonist ABT-737. We have carried our studies from model systems to the study of primary human malignancies. BH3 profiling and study of BCL-2 family member protein-protein interactions provide remarkably powerful and quantitative tools for the prediction of response to ABT-737 in lymphoid tumors. These assays are all the more useful as they require no *ex vivo* culture, and thus represent the apoptotic apparatus as it exists *in vivo*.

Questions:

BCL-2 expression is sufficient to confer addiction to BCL-2.
True
False

ABT-737 antagonizes BCL-2 by:
Decreasing transcription of the BCL-2 gene.
Decreasing translation of the BCL-2 trnascript.
Converting BCL-2 to a pro-death protein.
Binding to the hydrophobic cleft on BCL-2.

Primary CLL cells appear to be dependent upon for survival:
MCL-1
BCL-2
BIM

TRAIL-ARMED CD34+ CELLS AS SYSTEMIC VEHICLES OF CANCER GENE THERAPY

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Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is expected to play a key role in anti-cancer therapy due to its high cancer cell-specificity and potent antitumor activity. The clinical development of soluble (s)TRAIL is however hampered by several limitations, including (i) short plasma half-life; (ii) liver toxicity; (iii) tumor cell resistance. To overcome these limitations, we used CD34+ cells transduced with an adenovirus encoding the full-length human TRAIL gene (CD34-TRAIL+) as vehicles for intra-tumor delivery of membrane-bound (m)TRAIL. The mean (±SD) transduction efficiency of CD34+ cells exposed to a multiplicity of infection (MOI) of 500 was 83 ± 8% (range 70 – 95%) with a cell viability ≥85%. In vitro, exposure of the sTRAIL-sensitive KMS-11 cell line to CD34-TRAIL+, but not mock-transduced CD34+ cells consistently resulted in caspase-3, -8, and -9 activation and in PARP cleavage, as well as in potent induction of apoptosis (up to 80% after a 48-hour co-culture). Exposure of the sTRAIL-resistant JVM-2 cell line to CD34-TRAIL+ cells resulted in significant levels of tumor cell death (up to 50% after a 48-hour co-culture). Studies in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice xenografted with KMS-11 cell line showed that CD34-TRAIL+ cells significantly increased the median survival of mice bearing early-stage (92 vs 55 days, $P \leq 0.0001$) and advanced-stage (83 vs 55 days, $P \leq 0.0001$) disease, as compared with controls. Additionally, CD34-TRAIL+ cells significantly prolonged the median survival of mice xenografted with the sTRAIL-resistant JVM-2 (40 vs 31 days, $P \leq 0.0001$) and SU-DHL-4V (38 vs 30 days, $P \leq 0.0001$) cell lines. No obvious toxicity was observed upon administration of CD34-TRAIL+ cells. Histological analysis of subcutaneous lymphoma revealed an efficient tumor homing of transduced cells and high level expression of the agonistic TRAIL-R2 receptor by tumor endothelial cells. Following injection of CD34-TRAIL+ cells, but not mock-transduced CD34+ cells, TUNEL staining revealed increasing amounts of apoptotic cells with a 21-fold increase of the apoptotic index at 120 hour post-injection. Additionally, CD34-TRAIL+ cells induced signs of vascular damage leading to a progressive disintegration of the vascular bed, suggesting that tumor endothelial cells represent an early target of CD34-TRAIL+ cells. Our experiments show that: (i) *in vitro*, the co-culture of tumor cells and CD34-TRAIL+ cells resulted in a marked apoptosis of both sTRAIL-sensitive and sTRAIL-resistant tumor cells; (ii) in vivo, injection of CD34-TRAIL+ cells in mice bearing advanced-stage tumors as well as sTRAIL-resistant tumors was associated with a significant prolongation of survival. These results show that CD34-TRAIL+ cells might be an efficient vehicle for mTRAIL delivery to tumors, where they exert a potent antitumor effect possibly mediated by both direct tumor cell killing and indirect vascular-disrupting mechanisms.