Targeting alpha fetoprotein with SPEAR™ T-cells in hepatocellular carcinoma

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Introduction

Alpha fetoprotein (AFP), an oncofetal protein, is transcriptionally repressed after birth. Increased AFP in adult circulation is associated with liver regeneration, hepatitis, chronic liver diseases or malignant growth. Up to 50% of hepatocellular carcinoma (HCC) tumors express AFP and expression tends to be very high and homogenous, making AFP an attractive immunotherapy target.

We have generated an optimal affinity-enhanced T-cell receptor (TCR) specific for the HLA-A2-restricted peptide AFP116-185 (FMKFINFYED). AFP332, termed AFP SPEAR® T-cells. This will evaluate the safety and anti-tumor activity of AFP SPEAR® T-cells in HLA-A*0201+ patients with HCC, and good residual liver function.

Methods

As traditional animal models are not informative for human TCR safety studies, we have devised an extensive in vitro prescreening strategy, comprising three core components for assessing the specificity and potency of TCR engineered T-cells (Figure 1): Molecular mapping to identify potential cross-reactive peptides, verification of any identified peptides by loading candidates on antigen presenting cells and mass spectrometry to confirm candidate peptide processing and presentation.

1. Molecular mapping: If the TCR-peptide/MHC binding preferences to identify potential cross-reactive peptides, verification of any identified peptides by loading candidates on antigen presenting cells and mass spectrometry to confirm candidate peptide processing and presentation.

2. Human cell testing: against panels of primary normal cells from multiple organ systems in 2D, 3D and IPS (reduced pluripotent stem cell) culture formats to identify any cross-reactivities in more physiologically relevant cultures.

3. Potency testing: of the TCR transduced T-cells by antigen-driven proliferation, cytotoxicity and cytokine production.

Target Validation

1. Expression of AFP was assessed in normal hcc, HCC samples and other tumor cell lines. (Figure 2a).

2. Expression of AFP was liver restricted, with high and homogenous levels found in approximately one third of HCC samples and with some lower expression found in non-cancerous tissues.

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4. Expression of AFP was liver restricted, with high and homogenous levels found in approximately one third of HCC samples and with some lower expression found in non-cancerous tissues.

Cell screen

1. To screen for potential off-target cross-reactivity, AFP SPEAR® T-cells were tested by IFNγ secretion following incubation with HLA-A2 primary normal and tumor-derived human cells covering different cell types from a variety of organ systems (126 primary cells, 38 tumor lines). An example of the output against cells derived from one tissue system is shown in Figure 5. All primary normal cells and non-HCC cell lines were confirmed to be negative.

2. AFP SPEAR® T-cells recognized and secreted IFNγ in response to incubation with the AFP+ HCC cell line, HepG2, when compared to non-modified T cells. No response was detected by AFP SPEAR® T-cells against almost all cells of blood/immuno, brain/cerebral nervous system, cardiovascular, endothelium, gastrointestinal, reproductive, liver, musculoskeletal, respiratory or skin origin. Some responses were observed against these cell types; however, these were due to HLA-A*0201 alloreactivity or experimental aberrance.

In summary, no off-target AFP SPEAR® T-cell responses of concern were observed against a variety of cell types from a variety of organ system.

TCR generation and candidate selection

A TCR screening to screen for potential off-target cross-reactivity. AFP SPEAR® T-cells were assessed by IFNγ secretion following incubation with HLA-A2 primary normal and tumor-derived human cells covering different cell types from a variety of organ systems (126 primary cells, 38 tumor lines). An example of the output against cells derived from one tissue system is shown in Figure 5. All primary normal cells and non-HCC cell lines were confirmed to be negative.

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Potency

1. Cells grown in 2-dimensional culture may not represent the physiology and gene expression profile of cells grown in vivo, and therefore may not be entirely representative of the in vivo environment. Therefore, in addition to IFNγ secretion and cytokinase assays against HCC lines, we have included screening of AFP SPEAR® T-cells against more physiological cell lines including cells grown in 3D and against IPS cells.

2. Cytotoxic activity of AFP SPEAR® T-cells was only detected against 3D HepG2 HCC cell line microspheres (Figure 4) and not against AFP-negative A573 3D melanoma or N10 primary melanocyte microspheres, unless exogenous peptide was added to these cultures (data not shown). No response to IPS astrocytes, endothelial cells or cardiac myocytes was seen.

Conclusions

1. Target validation results indicated that AFP could be a very attractive target for HCC, with a potential therapeutic window for the TCR and a highly positive HCC tissue without marked recognition of non-cancerous tissue.

2. No safety concerns precluding clinical development were identified for AFP SPEAR® T-cell reactivity. 126 normal cells and 42 tumor cell lines from various organ systems were screened for AFP SPEAR® T-cells cytokine secretion and exhibited cytokinetic activity against HCC cell lines in 2D culture and 3D microspheres, but no relevant response to any other HLA-A0201-expressing cells.

3. Alloreactivity was detected against a subset of HLA-A0201 primary cells. A full alloreactivity screen performed using a panel of 51 EBV transformed B cell lines covering 38 HLA A, 63 HLA B and 28 HLA C alleles also demonstrated enhanced responses against HLA-A0204 and HLA-B01503 (data not shown). AFP SPEAR® T-cells did not recognize the AFP peptide in the context of HLA-A0203. Patients with HLA-A0204 and -B01503 and -A0202 will be excluded.

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