ImmunoTools *special* Award 2014

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Improving immune response to cancer vaccine for hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the third and the fifth leading cause of cancer-related death worldwide in men and women, respectively (http://globocan.iarc.fr/). The main risk factors for HCC are the hepatitis B and C virus (HBV and HCV) infections. Poor prognosis is due to the low efficacy of available treatments (1) and immunotherapeutic interventions targeting tumor-specific antigens may represent an alternative therapeutic tool (2).

However, a limited number of immunotherapy trials for HCC, including therapeutic vaccines, have been conducted with yet modest results (3,4,5). The first two HCC vaccine clinical trials have been conducted by Butterfield et al. based on $CD8^+$ T cell epitopes specific for a-Fetoprotein (AFP) administered directly as peptides or as autologous dendritic cells (DCs) loaded ex vivo. Generation of AFP-specific but only transient T cell responses in vaccinated subjects has been reported, possibly due to the lack of $CD4^+$ help (6). The other two HCC vaccine clinical trials have been based on autologous DCs pulsed ex vivo with a lysate of the autologous tumour (7) or of hepatoblastoma cell line HepG2 (8). Yet, both trials showed limited improvements in clinical outcomes.

The objectives of the research program will be to develop a highly innovative combinatorial cancer vaccine strategy aiming at improving the clinical outcome in early-stage hepatocellular carcinoma (HCC) patients after current standard resection or loco-regional therapy. In particular, the principal aim will be to improve the efficacy

of immunotherapies for HCC by generating a strong, specific, complete and long-lasting immunity against the tumor.

Currently, we are evaluating a novel combinatorial strategy based on chemotherapy plus vaccine in C57BL/6 mice. The chemotherapy is a multi-drug cocktail including taxanes and alkylating agents which is administered in a metronomic-like fashion. The vaccine is a multi-peptide cocktail including HCV epitopes, derived from NS3 and core viral proteins, as well as universal tumor antigen hTERT epitopes, adjuvanted in Montanide and CpG.

In particular, we are assessing the effect of daily metronomic chemotherapy, administrated in combination with peptide vaccine, evaluating kinetic of different T lymphocyte subsets including $CD4^+CD25^+$ Treg population. In parallel, we are evaluating the antigen-specific immune responses elicited in mice vaccinated with adjuvanted peptides in combination with daily metronomic chemotherapy. In particular, we are assessing the epitope specific activation of $CD4^+$ Th cells, IFNγ producing $CD8^+$ T cells, ex vivo CTL activity.

Preliminary data show that the combinatorial strategy induces an enhancement in eliciting specific T cell responses, when compared to adjuvanted peptides alone. Results of the designed combinatorial strategy indicates that the very low doses of chemotherapeutics included in the cocktail are effective in enhancing the cellular responses elicited by peptides. Such results are highly promising and may pave way to relevant improvement of immunotherapeutic strategies for HCC and beyond.

In parallel, we are studying the effects of such combinatorial strategy in an ex vivo vaccination platform (9, 10), targeting human PBMCs derived from healthy individuals as well as HCC patients. Activation markers as well as cytokine expression patterns are evaluated to assess the effects on human cells as prediction model for in vivo immunization.

Expansion and optimization of such studies are planned in the very next future and the selected ImmunoTools products would be of great benefit to this project.

1. Bruix J et al. 2014. Gut. 63: 844-855.
2. Buonaguro L et al. 2013. J Hepatol. 59: 897-903.
3. Reinisch W et al., 2002. J. Immunother. 25:489-499.
4. Sangro B et al., 2004. Clin. Oncol. 22:1389-1397.
5. Takayama T et al., 2000. Lancet 356:802-807.
6. Butterfield LH et al., 2006. Clin. Cancer Res. 12:2817-2825.
7. Lee WC et al., 2005. J. Immunother. 28:496-504.
8. Palmer DH et al., 2009. Hepatology 49:124-132.
9. Buonaguro L & Pulendran, B, 2011. Immunological Rev. 239:197–208.
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ImmunoTools *special* AWARD for **Maria Tagliamonte** includes 25 reagents

FITC - conjugated anti-human CD8,

PE - conjugated anti-human IFN-gamma,

PerCP - conjugated anti-human CD4,

APC - conjugated anti-human CD3,

human ELISA-set (for one 96 plate), human IFN-gamma, human IL-6, human IL-10, human TNF-a,

recombinant human cytokines: rh GM-CSF, rh IL-4, rh IL-6, rh TNFα, rh IL-1beta /IL-1F2, rh sCD40L / CD154, rh IL-2,

FITC - conjugated anti-mouse CD25,

PE - conjugated anti-mouse CD4 DETAILS more AWARDS