IMMUNOTHERAPY IN CANCER AND CHRONIC VIRAL INFECTION

We aim at ameliorating cytokine therapy in cancer and chronic viral infection with a focus on improved treatment efficacy in acute myeloid leukemia (AML) and more accurate tools for prognostication in chronic hepatitis C. Our previous studies have identified a mechanism of immunosuppression in cancer, which is mediated by myeloid cell-derived oxygen radicals. These toxic oxygen metabolites inactivate cytotoxic T cells and natural killer (NK) cells, and compounds that scavenge radicals or inhibit oxygen radical formation improve the immunostimulatory efficiency of T and NK cell-enhancing cytokines such as interleukin-2 (IL-2) or interferon-alpha (IFN-alpha). We have evaluated a radical formation inhibitor (histamine dihydrochloride, HDC) in preclinical models and as an adjunct to IL-2 in cancer therapy. Immunotherapy with HDC/IL-2 was found to maintain leukemia-free survival in AML patients in remission (n=320; p<0.008) in a recent phase 3 trial. Our results formed the basis for the approval of HDC (Ceplene®) by the European Commission in 2008; thereby, Ceplene became the first Swedish pharmaceutical to be approved for use within the EU in more than 10 years. In chronic hepatitis C we have found that a hepatocyte-derived chemokine, interferon-inducible protein-10, impacts on the early viral decay during treatment with IFN-alpha. The planned studies will assess (i) the molecular mechanisms of oxidant-induced apoptosis in lymphocytes, (ii) the role of NK and T cell functions for the occurrence of relapse in AML patients, (iii) the efficacy of HDC/IL-2 in related hematological malignancies, and (iv) the impact of IP-10 on the antiviral and immunomodulatory properties of IFN-alpha.

SCIENTIFIC PUBLICATIONS 2006-2009, Kristoffer Hellstrand (as indexed in PubMed)

SUMMARY

The scientific production during 2006-10 encompasses three major parts:

I. Molecular mechanisms of relevance to oxidative stress in lymphocytes

Paper 11 (Thorén et al., J. Immunol. 2007a) demonstrates that the CD56_{bright} subset of NK cells resists oxidative stress by consuming hydrogen peroxide and by expressing anti-oxidative glutathione. This finding, which was subsequently confirmed by other investigators (J. Immunol.179: 4513, 2007), implies that the immunoregulatory {bright} phenotype of NK cells, in contrast to the cytotoxic CD56_{dim} NK cell subset, is protected from oxidant stress.

Paper 15 (Thorén et al., J. Immunol. 2007b) demonstrates that myeloid dendritic cells, partly by scavenging oxygen radicals, confer protection of adjacent lymphocytes against oxidative stress. The paper was listed as a Research Highlight in Nat. Immunol. (8: 799, 2007), and broadens the understanding of dendritic cell/NK cell cross-talk. Specifically, the results may serve to explain how lymphocytes are protected from the oxidative damage associated with inflamed tissues.

Paper 21 (Thorén et al., J. Immunol. 2006) outlines the molecular mechanisms underlying oxidant-induced apoptosis in lymphocytes. The data demonstrate that exposure to oxidants results in over-activation of the nuclear PARP-1 DNA-repairing enzyme, which in turn leads to nuclear translocation of the mitochondrial apoptosis-inducing protein AIF. The apoptosis starts by AIF-mediated cleavage of DNA into larger fragments (50 kbp), and continues by
caspase-dependent DNA degradation into 150 bp fragments. In addition to contributing to the understanding of the molecular biology of oxidant-induced apoptosis, this paper proposes novel tentative approaches in rescuing lymphocytes from oxidative damage (figure 1).

**Figure 1.** Schematic representation of PARP 1-induced apoptosis in lymphocytes, with secondary activation of the caspase cascade. ROS = reactive oxygen species (paper 18).

**Paper 23** (Romero et al., Br. J. Haematol. 2006) shows that oxidant exposure results in the disappearance of the activating NKp46 and NKG2D receptors from the surface of viable NK cells. This finding was recently confirmed by others (J. Immunol. 182: 1696, 2009). The paper also shows that HDC efficiently restores NKp46 and NKG2D expression in NK cells. The latter finding is clinically interesting given that a deficient NKp46 expression predicts a high relapse risk in AML patients (Blood, 109: 323, 2007) and may shed further light on the mechanism of relapse protection achieved by HDC therapy in this disease (see paper 19).

**II. Clinical trials in cancer immunotherapy**

**Paper 13** (Middleton et al., Ann. Oncol. 2007) presents the final results of a phase III trial in metastatic melanoma (n=241) evaluating the clinical efficacy of combination therapy with IL-2/IFN-α/HDC vs. DTIC (a cytostatic commonly used in this disease). Median survival was longer for patients receiving IL-2/IFN/HDC (271 days) than for patients receiving DTIC (231 days), which did not achieve statistical significance. It is concluded that this immunotherapeutic regimen is not sufficiently active in metastatic melanoma.

**Paper 22** (Brune et al., Blood, 2006) presents the final results of a phase III trial in AML patients in remission (n=320) which evaluated the efficacy of low-dose IL-2 + HDC (HDC/IL-2) vs. standard of care (no treatment). The trial endpoint (leukemia-free survival at 3 years after the onset of therapy) demonstrated superiority of the HDC/IL-2 arm (log rank test p=0.008) with a more than 50% increase of the likelihood of relapse-free remission at 3 years. The results formed the basis for the approval of HDC as a relapse-preventive immunotherapy within the EU and other European countries in October, 2008.

**Papers 3 and 4** are reviews accounting for the preclinical background to the use of HDC in AML along with detailed clinical results.
III. Immune monitoring and prognostication in chronic hepatitis C

Papers 13 (Westin et al., Hepatology, 2007), 16 (Lagging et al., Hepatology 2006), and 17 (Romero et al., J. Inf. Dis, 2006) show that high systemic levels of the chemokine interferon-inducible protein-10 (IP-10) is correlated to a slow initial reduction of HCV-RNA during treatment of patients with chronic hepatitis C with interferon/ribavirin (i.e. the current standard-of-care). These findings have been confirmed by other groups (see e.g. Palmer et al. in the March, 2009 issue of Gut). Our most recent publication in this area (Askarieh et al., Hepatology 2010; paper 1) demonstrates that the prognostic value of IP-10 is confined to the viral decline during the first day of treatment. IP-10 may be useful as a supplementary tool to prognosticate the outcome of interferon therapy; in addition, these findings motivate an analysis of the effects of IP-10 on the antiviral and immunomodulatory properties of alpha-interferon, and may shed further light on the mechanisms of interferon resistance in chronic hepatitis C.

LIST OF PUBLICATIONS 2006-2009 (Kristoffer Hellstrand)
R = review, O = original article
Impact Factors according to JCR-Web 2007

1. Intrahepatic and systemic IP-10 determines the first day of viral decline during antiviral therapy with peg-interferon and ribavirin for chronic hepatitis C.
Hepatology (2010), in press.
Impact factor 10.7

2. Nonresponder patients with hepatitis C virus genotype 2/3 infection: a question of low systemic interferon concentrations?
Impact factor 6.7

3. Histamine dihydrochloride and interleukin-2 in acute myeloid leukemia (R + O).
Brune M, Romero AI, Hellstrand K.
Br. J. Pharmacol., 2009, in press
Impact Factor 3.8

4. Immunotherapy with histamine dihydrochloride and interleukin-2 in acute myeloid leukemia: background and results (R).
Hellstrand, K, Romero AI, Brune M.
Eur. Haematol., 2010, in press
Impact Factor 2.2

5. Are Foxp3^+ cells involved in hyporesponsiveness to interferon/ribavirin therapy in chronic hepatitis C?
Westin J, Hellstrand K, Dhillon AP, Lagging M.
J Viral Hepat. 2010
6. Weight-adjusted dosing of ribavirin and importance of hepatitis C virus RNA below 1000 IU/mL by day 7 in short-term peginterferon therapy for chronic genotype 2/3 hepatitis C virus infection (O).

Impact Factor 10.7

7. Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection (O).

Impact Factor 10.7

8. A non-invasive fibrosis score predicts treatment outcome in chronic hepatitis C virus infection (O).

Impact Factor 1.8

Dahlberg M, Dahlgren C, Hellstrand K, Movitz C.

Impact Factor 1.3

10. Wider indications for treatment of chronic hepatitis B? (R)
Lindh M, Hellstrand K, Norkrans G.

Impact Factor 3.0

11. The CD16-/CD56bright subset of NK cells is resistant to oxidant-induced cell death (O).
Thorén FB, Romero AI, Hermodsson S, Hellstrand K.

Impact Factor 6.1


Impact Factor 2.9

13. Results of a multicenter randomized study to evaluate the safety and efficacy of combined immunotherapy with interleukin-2, interferon-α2b and histamine dihydrochloride versus dacarbazine in patients with stage IV melanoma (O).
14. Response prediction and treatment tailoring for chronic hepatitis C virus gt 1 infection (O).
*Impact Factor* 3.7

15. Cutting edge: Antioxidative properties of myeloid dendritic cells: protection of T cells and NK cells from oxygen radical-induced inactivation and apoptosis (O).
Thorén FB, Betten A, Romero AI, Hellstrand K.
*Impact Factor* 6.1

16. Impact of disease severity on outcome of antiviral therapy in treatment-naïve patients with chronic hepatitis C (O).
*Impact Factor* 10.7

17. Impact of hepatic steatosis on viral kinetics and treatment outcome during antiviral treatment of chronic HCV infection (O).
*Impact Factor* 3.0

18. Predicting treatment outcome following 24 weeks peginterferon alpha-2a/ribavirin therapy in patients infected with HCV genotype 1: utility of HCV-RNA at day 0, 22, 29, and w 6 (O).
*Impact Factor* 10.7

19. IP-10 predicts viral response and therapeutic outcome in difficult-to-treat patients with HCV genotype 1 infection (O).
*Impact Factor* 10.7

20. Interferon (IFN)-gamma-inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFN-alpha 2a and ribavirin for chronic hepatitis C virus infection (O).
*Impact Factor 6.0*

Thorén FB, Romero AI, Hellstrand K.
*Impact Factor 6.1*

22. Improved leukemia-free survival after postconsolidation immunotherapy with histamine dihydrochloride and interleukin-2 in acute myeloid leukemia: results of a randomized phase 3 trial (O).
*Impact Factor 10.9*

23. NKp46 and NKG2D receptor expression in NK cells with CD56dim and CD56bright phenotype: regulation by histamine and reactive oxygen species (O).
Romero AI, Thorén FB, Brune M, Hellstrand K.
*Impact Factor 4.5*

**Completed PhD projects during the grant period**

*Winner of the Assar Gabrielsson Award (SEK 100,000) for best Ph D thesis in cancer research 2007*
*Holder of a VR-sponsored post-doc position in Laurence Zitvogel’s laboratory at INSERM, Paris 2008-10*

*Holder of an EMBO-sponsored post-doc position in Lorenzo and Alessandro Moretta’s laboratory at the Univeristy of Genova 2008-09*

**Ongoing doctorate projects (Ph D students)**

Post-docs

3. Fredrik Bergh Thorén, PhD
4. Ali Akhiani, PhD
5. Anna Martner, PhD

Research Grants (Principal Investigator K. Hellstrand)

The Torsten & Ragnar Söderberg Foundation 10,000,000 SEK (2008-2010)
Swedish Cancer Society (Cancerfonden) 1,000,000 SEK/year (2009-2011)
Swedish Medical Research Council (Vetenskapsrådet) 500,000 SEK/year (2009-2011)
Sahlgren’s University Hospital (LUA/ALF grant) 2,425,000 SEK/year (2008-2010)