Immunotherapy with Histamine Dihydrochloride and Interleukin-2 in Acute Myeloid Leukaemia

a report by

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In the initial phase of treatment in acute myeloid leukaemia (AML), patients receive chemotherapy with the intent to eradicate malignant blast cells from bone marrow and other tissues. Induction therapy usually induces the disappearance of microscopically detectable malignant cells (complete remission [CR]), and the ensuing consolidation therapy aims at preventing the subsequent recurrence of leukaemia (relapse). The refinement of induction and consolidation protocols has drastically improved AML prognosis over the past four decades,[1] but the occurrence of leukaemic relapse (typically within the first year after CR) is a major reason why the overall mortality of those diagnosed with AML remains worryingly high.

There are few therapeutic options available to prevent leukaemic recurrence. Patients may be subjected to allogeneic bone marrow transplantation (allo-BMT) from human leukocyte antigen (HLA)-matched siblings or unrelated donors, which efficiently reduces the relapse risk.[1] Fewer than 30% of AML patients are eligible for allo-BMT, and the standard of care after the completion of chemotherapy is observation for most patients. The prevention of relapse, which occurs in 60–70% of all non-transplanted AML patients in CR,[1] is a major challenge in AML. This article discusses the conceptual basis for immunotherapy with interleukin-2 (IL-2) in AML and reviews the results of clinical trials using IL-2 for relapse prevention. We also outline the rationale for combination immunotherapy with histamine dihydrochloride (HDC) and IL-2 as a post-consolidation remission maintenance strategy in AML.

Post-consolidation Immunotherapy with Interleukin-2 in Acute Myeloid Leukaemia

Two subsets of lymphocytes, T cells and natural-killer (NK) cells, are endowed with the capacity to recognise and destroy leukaemic cells. Immunotherapeutic regimens in AML have been developed to enhance the antileukaemic potential of these cytotoxic lymphocytes. The notion that T and NK cells participate in the surveillance of leukaemic cells in AML is supported by studies suggestive of a positive correlation between T/NK-cell function and a favourable disease outcome. This correlation is evident from studies in allo-transplanted patients, in whom T/NK-cells clearly mediate the graft-versus-leukaemia effect that forms the basis for relapse prevention.[1,2] A similar correlation has also been found in non-transplanted patients.[3–10] A therapy that improves antileukaemic functions of T and NK cells would have the potential to reduce the relapse risk in AML. The most frequently studied compound in this regard is the T- and NK-cell-activating cytokine IL-2.

IL-2, which is produced by T cells and is pivotal to several aspects of cell-mediated immunity, activates functions of T and NK cells such as antitumour cytotoxicity and production of cytokines (interferon-γ). In addition, it expands the population of T and NK cells by triggering cell-cycle proliferation. Beginning in the early 1990s, several single-arm trials were initiated using monotherapy with IL-2 to prevent relapse in the post-consolidation phase of AML. The results were considered encouraging. Subsequent controlled trials have yielded disappointing results. Five trials, the Blaise, CALGB 9720, CCG-2961, CALGB 19080 and ALFA 9801 trials, in a total of 905 patients have assessed the efficacy of IL-2 in prolonging leukaemia-free survival (freedom from relapse) versus the standard of care (no treatment) in AML patients in CR. None of these trials has shown significant beneficial effects of IL-2, given at monthly doses ranging between 25 and 91MIU,[11,15] on any parameter of clinical efficacy. In the CALGB 19080 trial, a trend towards a reduced relapse rate ($p>0.1$) was observed in younger AML patients in their first CR (CR1), but other trials in younger CR1 patients are not supportive of the relapse-preventative efficacy of IL-2 monotherapy.[14,15]

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Improvement of Interleukin-2 Efficiency by Histamine Dihydrochloride

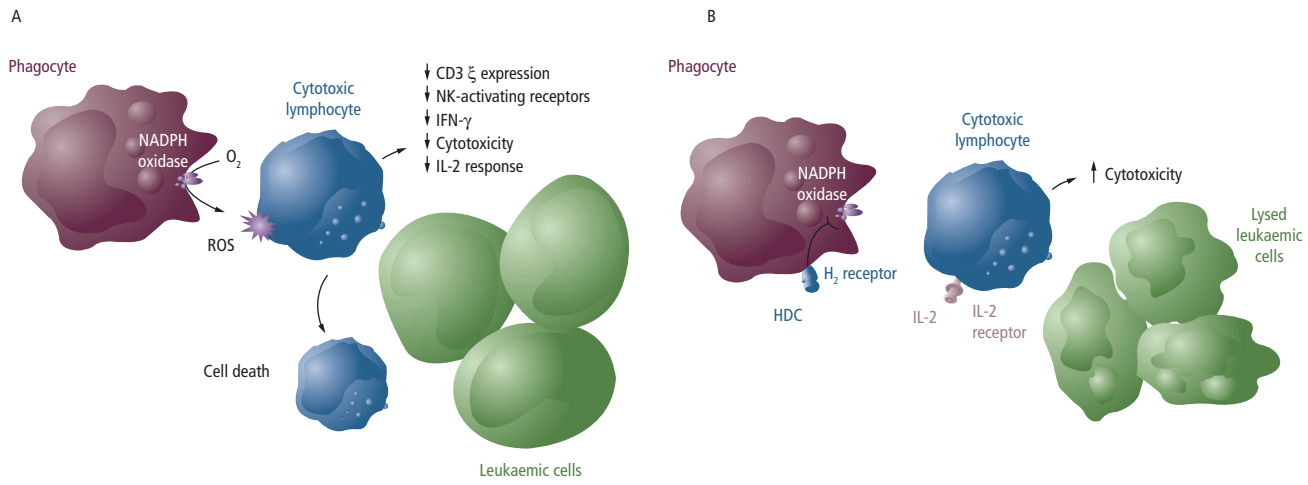
Mechanisms of Immunosuppression in Acute Myeloid Leukaemia

The mechanisms responsible for the poor clinical efficacy of IL-2 in AML are not known in detail, but it seems reasonable to ascribe the lack of efficiency to a suppressed state of IL-2's target lymphocytes in treated patients. In AML – and in several other forms of cancer – lymphocytes have been shown to be dysfunctional with, for example, a defective capacity to transduce activating signals and an increased propensity of undergoing apoptotic cell death.[16,17] This phenomenon – often referred to as 'cancer-related immunosuppression' and a significant part of the larger entity of 'cancer escape mechanisms' – may serve to explain not only why malignancies arise and progress, but also why the efficacy of immunotherapies such as IL-2 are frequently insufficiently efficacious in human cancer. A treatment that counters cancer-related immunosuppression may unravel a therapeutic potential of IL-2.

Table 1: Pre-clinical Pharmacodynamics of Histamine Dihydrochloride (HDC) and Interleukin-2 (IL-2) Protection by IL-2's Target Cells by HDC and Synergy Between HDC and IL-2

Reference	Focus of Study	Main Result(s)
Hellstrand et al., 1990[28]	Human NK-cell regulation	Synergy between HDC and IL-2 regarding NK-cell-mediated killing of cultured human and lymphoma cells leukaemic *in vitro*. Defines myeloid histamine H_2 receptors mediating effects of HDC.
Hellstrand et al., 1990[29]	Growth of NK-cell-sensitive tumours in mice	Synergy between HDC and IL-2 in inducing NK-cell-mediated destruction of melanoma cells *in vivo*.
Hellstrand et al., 1991[30]	Human NK-cell regulation	HDC potentiates IL-2-induced cell-cycle proliferation of human NK cells *in vitro* by countering a myeloid-cell-derived suppressive signal.
Hellstrand et al., 1994[20]	Human NK-cell regulation	Synergy between HDC and IL-2 regarding NK-cell-mediated killing of cultured human leukaemic and lymphoma cells *in vitro*. Defines the role of myeloid suppressor cells.
Hellstrand et al.,1994[31]	Human NK-cell regulation	Synergy between HDC and IL-2 in inducing antibody-dependent cellular cytotoxicity in NK-cells *in vitro*.
Asea et al., 1996[32]	Growth of NK-cell-sensitive tumours in mice	Synergy between HDC and IL-2 in inducing NK-cell-mediated destruction of YAC-1 lymphoma *in vivo*.
Asea et al., 1996[33]	Human NK-cell regulation	Synergy between HDC and IL-2 in inducing production of interferon-γ by NK cells *in vitro*.
Brune et al. 1996[27]	Human NK-cell regulation	Synergy between HDC and IL-2 in inducing NK-cell-mediated destruction of freshly recovered AML blasts *in vitro*. Defines the role of myeloid-cell-derived oxygen radicals.
Hansson et al., 1996[34]	Protection of NK cells	HDC-induced protection of NK-cells and T-cells against myeloid-cell-induced apoptosis *in vitro*.
Hansson et al., 1999[23]	Human T- and N-cell regulation	Synergy between HDC and IL-2 in inducing activation antigen expression (CD69) in T cells and NK cells *in vitro*.
Mellqvist et al., 2000[35]	Human T- and NK-cell regulation and protection of antileukaemic lymphocytes	Synergy between HDC and IL-2 in inducing NK-cell-mediated destruction of cultured leukaemic cells using polymorphnuclear myeloid cells or malignant CML cells as suppressor cells *in vitro*.
Betten et al., 2001[36]	Protection of T- and NK-cells against myeloid-cell-induced apoptosis	HDC protects T and NK cells against myeloid-cell-induced oxygen-radical-mediated apoptosis *in vitro*.
Kozar et al., ,2002[37]	Mouse NK-cell regulation	Potentiation by HDC of IL-2 induced activation of murine NK-cells *in vitro*.
Romero et al., 2006[38]	Human NK-cell regulation	HDC-induced preservation of activating NK-cell receptors (NKp46/NKG2D) *in vitro*.
Thorén et al., 2006[39]	Protection of antileukaemic lymphocytes	HDC-induced protection of NK cells and T cells against myeloid-cell-induced apoptosis *in vitro*. Defines the role of the PARP-1/AIF axis in myeloid cell-induced apoptosis.

Figure 2: Schematic Representation of Immunostimulatory Properties of Histamine Dihydrochloride

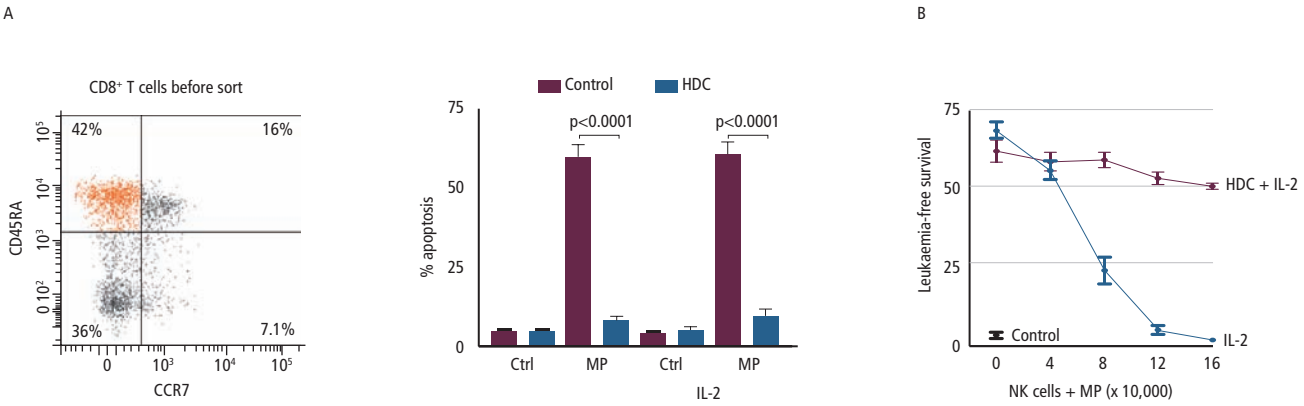


Panel A schematically describes how myeloid-cell-derived oxygen radicals inactivate antileukaemic lymphocytes and trigger apoptosis in these cells. Panel B shows the same scheme of events in the presence of histamine dihydrochloride (HDC), which inhibits oxygen radical production in myeloid cells and prevents these suppressive events. By maintaining the function and viability of antileukaemic lymphocytes, HDC synergises with the T- and NK-cell-activating cytokine interleukin (IL)-2 to induce killing of sensitive target cells, including human acute myeloid leukaemia blasts.

Clinical Pharmacodynamics of Histamine Dihydrochloride

During the course of the study using HDC/IL-2 in cancers other than AML, the effect of treatment with HDC/IL-2 on T- and NK-cell functions was monitored and compared with that of IL-2 monotherapy. In melanoma, patients receiving HDC/IL-2 showed improved induction of IFN-γ in several types of T cell,[24] and in renal cell carcinoma, treatment with HDC/IL-2 was associated with improved functional status of cytotoxic lymphocytes in peripheral blood and at the site of malignant tumour growth.[25]

Figure 3: Histamine-dihydrochloride-induced Protection of Differentiated Human Cytotoxic T Cells and NK-cell Cytotoxicity Assay



Panel A shows an example of the histamine dihydrochloride (HDC)-induced protection of differentiated human cytotoxic T-cells (with CD45RA$^+$/CCR$^-$/CD8$^+$ phenotype); these cells are induced to apoptosis by mononuclear phagocytes, and protected in the presence of HDC.[26] Panel B shows the results of an NK-cell cytotoxicity assay using freshly recovered human acute myeloid leukaemia (AML) blasts as target cells. As shown, interleukin (IL)-2 efficiently induces killing of these target cells in the absence of mononuclear phagocytes. This cytotoxic response is reduced or inhibited by adding increasing amounts of mononuclear phagocytes. HDC abrogates the mononuclear-cell-induced suppression and synergises with IL-2 to induce NK-cell-mediated lysis of AML blasts.[27]

A tentative conclusion from these clinical pharmacodynamic studies is that treatment of cancer patients with HDC/IL-2 improves T- and NK-cell functions more efficiently than IL-2 alone, as predicted in pre-clinical models. These findings are considered relevant to the action of HDC and IL-2 in AML, since the studies demonstrate that HDC improves the efficacy of IL-2 on lymphocyte subsets that have been ascribed a role in the maintenance of leukaemia-free survival in AML. To clarify whether HDC improves the immune activation by IL-2 in AML patients, comparisons of the immunostimulatory properties of HDC/IL-2 versus IL-2 were performed during an initial phase II trial.[21] Blood samples were drawn during an initial cycle of IL-2 monotherapy, and the level of accumulation of NK cells and activated T cells was compared with that achieved during a subsequent cycle with HDC/IL-2. The results showed a non-significant trend towards improved NK-cell expansion during the cycle with HDC/IL-2, along with a significantly more pronounced accumulation of activated T-cells (p=0.003; n=7) compared with IL-2 monotherapy. Although obtained in a small number of patients, these findings support that the HDC-induced potentiation of the immunostimulatory efficacy of IL-2 observed in other histotypes of cancer also occurs in AML.

Conclusions

Despite an impressive pre-clinical rationale, monotherapy with the T- and NK-cell-activating cytokine IL-2 has so far yielded disappointing results in clinical trials aimed at reducing the high frequency of relapse in patients with AML. The use of HDC in conjunction with IL-2 for relapse prevention in AML was founded on pre-clinical studies demonstrating that HDC improves the T- and NK-cell-activating properties of IL-2, specifically by countering suppressive signals from adjacent myeloid cells. Several functions of T and NK cells are improved by the addition of HDC to IL-2, and clinical pharmacodynamic studies support that a similar improvement of the immunostimulatory efficiency of IL-2 also occurs during treatment of patients with HDC added to IL-2. The results of a recent phase III trial demonstrate the superiority of HDC/IL-2 over the current standard of care in preventing relapse in AML patients. The treatment appears to be particularly efficacious in CR1 patients and in patients below 60 years of age. These findings imply that the countering of cancer-related immunosuppression may improve the clinical efficacy of IL-2 and may form the basis for the further development of immunotherapy as a relapse-preventative strategy in AML. ■

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