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Is there a role for immunotherapy in Hodgkin's disease?

To date, most patients with Hodgkin's lymphoma (HL) can be cured with chemotherapy, radiotherapy or combined modality treatment. However, current treatment is associated with severe side effects and late toxicities such as infertility, cardiovascular damage and secondary malignancies. Moreover, a fraction of patients suffers from refractory disease and cannot be cured with current approaches including high dose chemotherapy and stem cell transplant.

In HL cellular and soluble tumor-cell derived factors CD30/sCD30, TARC and others inhibit local and systemic anti tumor immunity. In addition, there is a specific expression of tumor cell antigens such as CD30, CD15 and CD25 on malignant Hodgkin-Reed-Sternberg cells (HRS cells) making HL an ideal candidate for immunotherapeutic strategies.

Current immunotherapeutic approaches include unconjugated monoclonal antibodies, radioimmunconjugates, immunotoxins and – very recently – novel immunomodulatory compounds.

This talk will summarize current and novel approaches in the immunotherapy of Hodgkin`s Lymphoma and discuss its possible role in the future.

Unconjugated anti-CD30 antibodies

CD30 is a member of the tumor necrosis factor receptor (TNFR) superfamily and is almost exclusively expressed by the malignant Hodgkin-Reed-Sternberg cells in Hodgkin`s Lymphoma. CD30 activation has pleiotropic effects depending on the cell line and the cellular activation status and can induce apoptosis or pro-proliferative signaling.[1] Monoclonal anti-CD30 antibodies were shown to induce complement-dependent and antibody-dependent cytotoxicity and had promising activity *in vitro* and in xenograft models of human HL.[2-4] Current clinical trials focus on the chimeric humanized SGN-30 (Seattle Genetics) and the fully humanized MDX-060 (5F11, Medarex).

A first phase I single-dose trial showed minimal toxicity associated with doses of 1-15 mg/kg SGN-30 and moderate antitumor activity with responses in 2 of 13 treated patients.[5] Subsequently, SGN-30 was tested in a phase I/II multidose study in 24 patients with refractory or relapsed HL or $CD30^+$ non-Hodgkin-Lymphoma (NHL). Patients received 6 weekly doses of intravenous SGN-30 at 4 dose levels (2, 4, 8, or 12

mg/kg). SGN-30 was generally well tolerated and the MTD was not reached in this study. One complete remission was observed in a patient with Anaplastic Large Cell Lymphoma. However, activity in HL patients was somewhat disappointing with four patients achieving stable disease duration ranging from 6 to 16 months and no PRs or CRs.[6]

Similarly, the fully humanized anti-CD30 antibody MDX-060 was tested for safety in a phase I/II open-label, dose-escalation study in patients with relapsed or refractory Hodgkin's Lymphoma or CD30$^+$ NHL without any dose-limiting toxicities occurring. Consequently, a phase II trial was done with patients receiving MDX-060 at 10 or 15 mg/kg. MDX-060 was tolerated well in this study, serious adverse events occurred in five patients. There was moderate clinical activity, responses were observed in four of the 63 treated HL patients including 2CRs and 2PRs.[7]

In conclusion, unconjugated anti-CD30 antibodies are well tolerated in HL showing only moderate activity in refractory and relapsed patients. Since *in vitro* data suggests favorable combination activity with conventional chemotherapeutics, future clinical trials could evaluate combination strategies.

Immunotoxins

Immunotoxins or antibody-drug-conjugates consist of a specific antibody linked to a cytotoxic agent. Few of the numerous preclinically developed constructs have been tested in clinical trials.

Ricin A, a ribosome inactivating toxin, was used as an effector in phase I studies in relapsed and refractory patients linked to either an anti-CD25- or an anti-CD30-directed antibody.[8,9] Both constructs were moderately toxic with vascular-leak syndrome being the most relevant sign of toxicity and in both studies approximately 40-50% of the patients produced human-anti-ricin-antibodies (HARA) in response to the treatment. Reponse rates were comparable in both studies with ORR (overall response rate) around 15% and no CRs observed.

Other immunotoxins include an anti-CD30-saporin construct which was tested in two studies in 12 patients overall resulting in some activity (four PRs, three MRs);[10] and an anti-CD25-pseudomonas-exotoxin-A construct with moderate activity.[11]

Very recently, Younes and colleagues reported preliminary results of an ongoing phase I dose-escalation trial in relapsed and refractory HL patients with 0.1-3.6 mg/kg SGN-35, an anti-CD30-antibody- monomethylauristatin E (MMAE) conjugate. SGN-35 was designed to be stable in the blood and to release MMAE only upon internalization into CD30-expressing tumor cells. 36 HL patients have been treated so far and SGN-35 was reported to be generally well tolerated.

In these heavily pretreated patients, a total of five CRs and seven PRs were reported which occurred in the higher dose levels, fifteen patients had stable disease.

Radioimmunotherapy

Since HLs are highly sensitive to radiation, radioimmunotherapy appears to be a promising approach. In addition, radiolabeled antibodies consisting of a tumor-targeted specific antibody labeled with an alpha- or beta-emitter have the advantage of killing not only the tumor cells but also cells of the microenviroment that might be substantial for tumor growth (cross fire effect).

In early clinical trials, Yttrium90- or Iodine131-labeled polyclonal antiferritine antibodies were tested including myeloablative and non-myeloablative strategies in multiple

relapsed and refractory HL patients. This approach led to remission rates around 20-80% depending on schedule and administered dose with considerable mostly hematological toxicity (reviewed in reference[5]).

In a recently published phase I trial, 22 relapsed or refractory patients were treated with murine Iodine131-labeled anti CD30 (Ki4) antibody resulting in an ORR of 27% including one CR, five PRs and three minor responses. The median duration of response was four months and the most significant toxicity was severe myelosuppression consisting of grade IV hematotoxicity 3-5 weeks after treatment in 8 patients.[12]

Anti CD20-antibody rituximab

HL can be classified into classical HL (cHL) the clinically and histologically distinct subtype Lymphocyte-predominant Hodgkin`s Lymphoma (LPHL), the latter accounting for about 5% of all HL cases13. In contrast to cHL, LPHL typically shows strong expression of CD20 along with CD30 and CD15 in the malignant cell population. Clinically, LPHLs show a more indolent course compared to cHL and patients mostly present in early stages of the disease. Treatment of LPHL using standard HL protocols is highly effective leading to complete remission in more than 95% of patients13. Given the indolent course of the disease, LPHL patients are likely to be overtreated with current cHL-derived protocols. Particularly early stage LPHL patients have an excellent prognosis and are more likely to die from treatment-associated late toxicities than from the disease itself.[14]

Given the indolent clinical course and the strong CD20 expression in LPHL tumors, the monoclonal anti-CD20 antibody Rituximab was suggested to be a non-mutagenic, less toxic alternative treatment for LPHL. The German Hodgkin Study Group (GHSG) recently reported the results of a phase II trial of rituximab (375 mg/m^2 in 4 doses) in 21 relapsed or refractory NLPHL patients.[15] 15 of the 21 enrolled patients were evaluable with confirmed diagnosis of LPHL and were analyzed resulting in an overall response rate of 94%, including 8 patients with complete remission (CR) and 6 patients with partial remission (PR). With a median follow-up of 63 months (range 3-84), the median time to progression was 33 months, with the median overall survival (OS) not reached. Similar results were reported from another study of standard dose Rituximab in 22 patients with relapsed LPHD resulting in an ORR of 100% with CR in 41%, CRu 5% and PR in 54%.[16]

In conlusion, these highly promising results suggest a change of paradigm in the treatment of LPHL; however, further investigation is warranted.

In classical HL, the malignant cells express CD20 in only about 25-30% of cases. However, CD20 is strongly expressed by the tumor infiltrating B-cells which contribute to HL tumor growth by providing antiapoptotic signals for the malignant HRS cells via CD30-CD30L and CD40-CD40L crosstalk.[17] Moreover, recent findings suggest that similar to mechanisms observed in leukemias and solid tumors, malignant HRS cells in HL tumors might arise from a CD20$^+$ "Hodgkin`s Stem Cell". Based on this rationale, 22 patients with relapsed CD20$^+$ or CD20$^-$ cHL were treated with weekly infusions of 375 mg/m^2 Rituximab in a pilot study. Responses were observed in 22% of patients with CR in one patient. Interestingly, responses were seen in both, CD20$^+$ and CD20$^-$ HL cases.

These results and the low rate of adverse effects associated with Rituximab have led to the implementation of Rituximab in combination into large first-line trials in Hodgkin`s Lymphoma. The recently initiated HD18 trial

for advanced stage cHL from the GHSG will evaluate the efficacy of Rituximab in combination with the dose-escalated BEACOPP regimen.

Adoptive Immunotherapy and bispecific antibodies

Eppstein Barr Virus (EBV) antigens EBNA1, LMP1 and LMP2 can be detected in HRS cells of approximately 40% of the HL cases18. Since no effective cellular anti-EBV response is observed in these patients, *in vitro* generation and subsequent administration of autologous EBV-specific T-cells has been tested in pilot studies and phase II clinical trials.[19,20] In the most extensive study, Bollard and colleagues treated 14 relapsed or refractory patients who had measurable disease or were considered at high risk for relapse with (LMP2-) EBV-specific T-cells. They observed *in vivo* expansion and anti-viral activity of EBV-specific T-cells and reported some activity including complete remissions.[19] Unfortunately, targeting EBV is limited to the EBV+ HL cases and the generation of EBV-specific T-cells is time consuming and complex, which might be obstacles to the clinical application.

Bispecific monoclonal antibodies simultaneously bind to the tumor cells, and, with a second binding site, to an immune effector cell. Until now, bispecific constructs using NK-cells and monocytes/macrophages as effector cells have been tested in clinical trials. The murine Bi-MAb HRS-3/A9 binds to CD30 on HRS cells and CD16 on NK cells and was tested in a phase-I/II study in 15 patients with relapsed or refractory HL. HRS-3/A9 was well tolerated in this study without reaching the MTD and responses included one CR, one PR and three MRs.[21] In an attempt to further activate the effector NK-cells, HRS-3/A9 was co-administered with IL-2 and GM-CSF in a second trial involving 16 refractory patients.[22] Response included one CR and three PRs with comparable tolerability.

A bispecific construct containing CD30-binding Ki-4 fragments and domains binding CD64 expressed on human monocytes was tested in a phase I dose-escalation trial in ten patients with relapsed or refractory HL. The MTD was not reached, and side effects were moderate. Responses observed included one CR and three PRs; four patients had stable disease.[23]

As novel class of compounds, the immunomodulatory drugs (IMIDs) thalidomide and its derivative lenalidomide have been approved for the treatment of hematological malignancies as multiple myeloma and myelodysplastic syndrome.[24] Besides possessing anti-angiogenic and possibly direct anti-tumor-cell-activity, these IMIDs stimulate T cell proliferation and enhance NK cell function via IL-2 and IFN-γ.[25] The alliance of direct anti-tumor activity with anti-angiogenic and immunostimulatory properties was the rationale for the treatment of multiple relapsed and refractory HL patients in a named patient program in Cologne and other German sites. Although these are only case experiences, a number of complete remissions observed in this group of heavily pretreated patients has led to the initiation of clinical trials testing the activity of lenalidomide in HL.

Conclusions and perspective

Different immunotherapeutic concepts have been tested for the treatment of Hodgkin`s Lymphoma including unconjugated monoclonal antibodies, immunotoxins, radioimmunoconjugates and, most recently, immunomodulatory compounds. Some strategies, in particular radioimmunotherapeutic

approaches and immunotoxins have already shown significant effectivity. First experiences with relatively non-toxic immunomodulatory compounds have implemented a whole new kind of immunotherapy in or LPHL the anti-CD20-antibody Rituximab might be the future effective but less toxic treatment. Therefore, the question is not whether there is a role for immunotherapy in HL but how immunotherapeutic concepts can be implemented into the current treatment concepts the best way in order to conserve or even improve the good long term survival in HL patients and to reduce toxicity and long-term side-effects. One challenge for the development of any new concept remains the recruitment of patients in clinical trials since HL is a rare disease and the majority of patients can be cured with conventional chemotherapy. International cooperations could be the solution to this problem.

References

1. Horie R, Higashihara M, Watanabe T. Hodgkin's lymphoma and CD30 signal transduction. Int J Hematol 2003;77:37-47.
2. Boll B, Hansen H, Heuck F, et al. The fully human anti-CD30 antibody 5F11 activates NF-{kappa}B and sensitizes lymphoma cells to bortezomib-induced apoptosis. Blood 2005;106:1839-42.
3. Borchmann P, Treml JF, Hansen H, et al. The human anti-CD30 antibody 5F11 shows in vitro and in vivo activity against malignant lymphoma. Blood 2003;102:3737-42.
4. Cerveny CG, Law CL, McCormick RS, et al. Signaling via the anti-CD30 mAb SGN-30 sensitizes Hodgkin's disease cells to conventional chemotherapeutics. Leukemia 2005;19:1648-55.
5. Klimm B, Schnell R, Diehl V, Engert A. Current treatment and immunotherapy of Hodgkin's lymphoma. Haematologica 2005;90:1680-92.
6. Bartlett NL, Younes A, Carabasi MH, et al. A phase 1 multidose study of SGN-30 immunotherapy in patients with refractory or recurrent CD30+ hematologic malignancies. Blood 2008;111:1848-54.
7. Ansell SM, Horwitz SM, Engert A, et al. Phase I/II study of an anti-CD30 monoclonal antibody (MDX-060) in Hodgkin's lymphoma and anaplastic large-cell lymphoma. J Clin Oncol 2007;25:2764-9.
8. Engert A, Diehl V, Schnell R, et al. A phase-I study of an anti-CD25 ricin A-chain immunotoxin (RFT5-SMPT-dgA) in patients with refractory Hodgkin's lymphoma. Blood 1997;89:403-10.
9. Borchmann P, Schnell R, Engert A. Immunotherapy of Hodgkin's lymphoma. Eur J Haematol Suppl 2005:159-65.
10. Falini B, Bolognesi A, Flenghi L, et al. Response of refractory Hodgkin's disease to monoclonal anti-CD30 immunotoxin. Lancet. 1992;339:1195-1196.
11. Kreitman RJ, Wilson WH, White JD, et al. Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. J Clin Oncol 2000;18:1622-36.
12. Schnell R, Dietlein M, Staak JO, et al. Treatment of refractory Hodgkin's lymphoma patients with an iodine-131-labeled murine anti-CD30 monoclonal antibody. J Clin Oncol 2005;23:4669-78.
13. Nogova L, Reineke T, Brillant C, et al. Lymphocyte-predominant and classical Hodgkin's lymphoma: a comprehensive analysis from the German Hodgkin Study Group. J Clin Oncol 2008;26:434-9.
14. Diehl V, Sextro M, Franklin J, et al. Clinical presentation, course, and prognostic factors in lymphocyte-predominant Hodgkin's disease and lymphocyte-rich classical Hodgkin's disease: report from the European Task Force on Lymphoma Project on Lymphocyte-Predominant Hodgkin's Disease. J Clin Oncol 1999;17:776-83.
15. Schulz H, Rehwald U, Morschhauser F, et al. Rituximab in relapsed lymphocyte-predominant Hodgkin lymphoma: long-term results of a phase 2 trial by the German Hodgkin Lymphoma Study Group (GHSG). Blood 2008;111:109-11.
16. Ekstrand BC, Lucas JB, Horwitz SM, et al. Rituximab in lymphocyte-predominant Hodgkin disease: results of a phase 2 trial. Blood 2003;101:4285-9.
17. Re D, Kuppers R, Diehl V. Molecular pathogenesis of Hodgkin's lymphoma. J Clin Oncol 2005;23:6379-86.
18. Re D, Thomas RK, Behringer K, Diehl V. From Hodgkin disease to Hodgkin lymphoma: biologic insights and therapeutic potential. Blood 2005;105:4553-60.
19. Bollard CM, Aguilar L, Straathof KC, et al. Cytotoxic T lymphocyte therapy for Epstein-Barr virus+ Hodgkin's disease. J Exp Med 2004;200:1623-33.
20. Roskrow MA, Suzuki N, Gan Y, et al. Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for the treatment of patients with EBV-positive relapsed Hodgkin's disease. Blood 1998;91:2925-34.
21. Hartmann F, Renner C, Jung W, et al. Treatment of refractory Hodgkin's disease with an anti-CD16/CD30 bispecific antibody. Blood 1997;89:2042-7.
22. Hartmann F, Renner C, Jung W, et al. Anti-CD16/CD30 bispecific antibody treatment for Hodgkin's disease: role of infusion schedule and costimulation with cytokines. Clin Cancer Res 2001;7:1873-81.
23. Borchmann P, Schnell R, Fuss I, et al. Phase 1 trial of the novel bispecific molecule H22xKi-4 in patients with refractory Hodgkin lymphoma. Blood 2002;100:3101-7.
24. Maier SK, Hammond JM. Role of lenalidomide in the treatment of multiple myeloma and myelodysplastic syndrome. Ann Pharmacother 2006;40:286-9.
25. Davies FE, Raje N, Hideshima T, et al. Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple myeloma. Blood 2001;98:210-6.