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REVIEW

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Clinical perspectives of bispecific antibodies in cancer

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Introduction

The bispecific antibody (bsAb) field has come of age, but definite "proof of concept" in clinical studies is still eagerly awaited. The 5th World Conference on Bispecific Antibodies at Volendam, the Netherlands, is expected to unveil several of these studies. One of the main aims of bsAb is to develop alternatives or adjuncts for chemo- and radiotherapy in cancer. We will give a personal perspective on the treatment of cancer, in which the choice of both the effector cells and the target antigen is of major importance.

Format of the bsAb

The first bsAb constructs have been made by biochemical cross-linking of (mainly Fab fragments of) IgG molecules. Whereas this is a fast procedure, for clinical applications these constructs harbour some disadvantages, such as the possible introduction of neoantigens and limited yield per batch with intrinsic batch-to-batch variations. bsAb may also be made biologically, via fusion of two established hybridoma clones. These so-called hybrid hybridomas harbour the genetic information of both parental antibody-producing clones; apart from the desired bsAb product, the parental mAb will be produced as well, and even a large variety of mismatch (H/L) molecules may emerge. Isolation of the desired bsAb represents a daunting task. Further, the mouse-derived hybridoma clones may have to be "humanised", in order to minimize (but be shown not to prevent) human antibody formation, hampering consecutive treatment. Both problems (production and human Ab formation) may be obviated by the application of so-called, phage Ab, which can be derived from a human library, and which may be made in various formats, including bispecific "diabodies."

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Table 1 Different types of effector cells and their target molecules

Effector cell	Target molecule on effector cell
T cell	CD 3 [1, 2, 9, 12]
NK cell	CD16 [8, 19]
Monocyte/macrophage	CD64 [4, 17]
Dendritic cell	CD64 [6]
Granulocyte (activated)	CD64 [5, 15, 16] CD89 [11]

Effecter cells in cancer treatment

The approaches up to now involve five different effector cells (Table 1).

It is not yet clear which effector cell will be the most effective, and this may depend on the type of tumour (haematological tumours or solid tumours) and the ultimate aim (direct antitumour effect or the development of T cell immunity against the tumour). We surmise that, in haematological tumours, especially B cell malignancies, T cells will be more effective because of the intimate contact between T cells and B cells in the normal immune response. In solid tumours natural killer (NK) cells, macrophages and granulocytes may be more effective. NK cells and granulocytes may be more directly effective, whereas T cells and macrophages/dendritic may be more effective in developing antitumour immunity. If this is true the ultimate application may even involve a sequential use of these bispecific antibodies (retargeting different effector cells). Remarkably, in several studies, it has been clear that

Table 2 Additional activation required by effector cells. *IL-2* interleukin-2, *GM-CSF* granulocyte/macrophage-colony-stimulating factor, *G-CSF* granulocyte-colony-stimulating factor

Effector cell	Concomitant/preceding activation
T cell	CD28 mAb, CD28 bsAb, IL-2 [3, 13, 18]
NK cell	high dose IL-2
Granulocyte	G-CSF [5, 15, 16]
Macrophage	GM-CSF [7]

additional activation of these effector cells is necessary (Table 2).

Choice of antigen on tumour cells

There seems to be a great difference here between haematological and solid tumours, as the normal counterparts of the haematological tumours, e.g. B cells in B cell malignancies, can be eliminated without problem, as they are replaced from stem cells after a few weeks. In solid tumours, specific target molecules, not expressed on the normal counterparts and recognisable by antibodies, are scarce. However, on solid tumours several oncogenic proteins are expressed in a much higher density than in normal cells and are more accessible to effector cells, e.g. HER-2/neu, EGF-R.

Clinical studies

Up to now several phase I and phase II studies have been performed, showing limited toxicity of the various bsAb in phase I studies. In these and subsequent phase I/II or phase II studies some efficacy has been shown against the tumour in patients with advanced disease in terms of response. However, it is questionable whether the standard criteria for response are useful for objective evaluation of the effect of immunotherapy in these patients. It is generally assumed that immunotherapy will be most effective in minimal residual disease and therefore we probably have to be satisfied with freedom from progression in patients with advanced disease. At least it must not divert us from the ultimate aim of treating residual disease. A summary of these studies is given in the Table 3.

Conclusion

The bsAb field has reached a critical stage and now needs to prove its efficacy in clinical studies. Essential for the eventual realisation of phase II/III studies is the availability of a standardised product that can be made in large amounts. Therefore recombinant products and good production methods are necessary, as well as their evaluation in animal models and comparison to traditional/complete bsAb. Meanwhile other applications outside the cancer field will surely stimulate the progress we anticipate to see on the 5th World Conference at Volendam.

Table 3 Summary of immunotherapy studies

Tumour	Tumour-associated antigen
B cell malignancies	CD19 [2]
Hodgkin's disease	CD30 [8, 13]
Breast/ovarian cancer	HER-2/neu [17, 19]
Ovarian cancer	Mov-18 [1]
Squamous cell cancer	EGF-R
Colorectal cancer	17-1A/EGP-2 [10, 14)
Renal cell cancer	17-1A/EGP-2 [9]

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