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Consensus

Expert opinions on endocrine toxicity induced by new anticancer therapies: Precautions to be taken in performing and interpreting hormonal assays under immunotherapy

*Avis d'experts sur la toxicité endocrinienne des thérapies anti-cancéreuses: analyse et interprétation
des dosages biologiques*

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Abstract

As well as tyrosine kinase and mTOR inhibitors, new anticancer therapies make use of antibodies targeting tyrosine kinase receptors or blocking anti-tumor immune response checkpoints. These are always monoclonal; in their international non-proprietary names, the origin is prefixed to “-mab”: e.g., mouse antibodies end in “-o-mab”, chimeric antibodies in “-xi-mab”, humanized antibodies in “-zu-mab” and human antibodies in “-u-mab”. When the analytic principle of the assay involves a murine monoclonal antibody and the therapeutic antibody contains a murine sequence, analytic interference is to be feared if the patient develops antibodies against the therapeutic antibody. The interfering heterophilic antibody may be a HAMA (anti-mouse), a HACA (anti-chimeric) or a HAHA (anti-humanized-antibody). In immunoassay for patients under immunotherapy, it is therefore recommended to check the type of therapeutic antibody: if it is liable to contain murine sequences, heterophilic antibodies should be screened for and neutralized.

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1. Introduction

New anticancer therapies are founded on very different principles from those of conventional chemotherapy; this is true for tyrosine kinase inhibitors, mTOR inhibitors and monoclonal antibodies targeting a specific tumor-cell protein. There are presently some 15 tyrosine kinase inhibitors: their international non-proprietary names (INN) end in “-ib” or “-inib”. The mTOR protein is a specific serine-threonine kinase protein regulating cell growth and survival. These inhibitors, used in a large

number of solid cancers, have INNs ending in “-rolimus” or “-ib”. It is important to bear in mind that tyrosine kinase and mTOR inhibitors are not proteins and thus do not induce production of heterophilic antibodies.

Such is not the case of the antibodies used in anticancer immunotherapy, whether targeting tyrosine kinase receptors or blocking anti-tumor immune response checkpoints, which can induce production of heterophilic antibodies liable to cause analytic interference in immunoassays. This risk depends both on the type of antibody and on the technique employed.

2. Production of therapeutic monoclonal antibodies (mab)

2.1. Mouse antibodies (o-mab: the “o” in “mouse” indicating the origin)

Since the studies by Kohler and Milstein [1], it has been possible to produce murine monoclonal antibodies by fusing mouse B cells, immunized against a given foreign protein, and myeloma cells able to proliferate indefinitely.

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This type of antibody has been used therapeutically since 1981. The drawback is that, after administration, they systematically induce production of heterophilic human anti-mouse antibodies (HAMA), partially neutralizing the therapeutic antibody, and sometimes even showing harmful effects.

They are thus not systematically associated with symptoms, but HAMA in a sample produces artifactual interferences in immunoassays using murine antibody reagents, as explained below.

This class of antibodies includes Bexxar[®], Blincyto[®], Orthoclone-Okt3[®], Removab[®], and Zevalin[®].

2.2. Chimeric antibodies (xi-mab: “xi” corresponding to the “chi” of “chimera” in Greek)

In the 1980s, molecular engineering succeeded in creating chimeric human/mouse antibodies (see [2] for review) by fusing genes coding for variable regions of a mouse antibody and constant regions of human immunoglobulin. They conserve the specificity and affinity of the murine parent antibody, with the advantage of having a much longer half-life and being better tolerated. Xi-mab treatment induces fewer pathologic side-effects, but production of anti-mouse antibodies may nevertheless occur, and was reported to be intense in 40% of patients [3]. This is important for biologists: the probability of HAMA interference in the immunoassay is less than with o-mab, but still exists.

This class of antibodies includes Adcetris[®], Erbitux[®], Flixabi[®], Inflectra[®], Mabthera[®], Remicade[®], Remsima[®], Rituxan[®], Rixaton[®], Sylvant[®], and Truxima[®].

2.3. Humanized antibodies (zu-mab: “zu” corresponding to the “z” and the “u” of “humanized”)

Humanization consists in transferring amino acids from complementary determining regions (CDR) of a murine monoclonal antibody (donor) to a human acceptor [4]. Obviously, the most appropriate human antibody has to be selected, so that most of the amino acids of the scaffolding region are identical between acceptor and donor. Humanized antibodies thus contain at least some specifically murine sequences; however, some 10% of patients receiving humanized antibodies develop anti-humanized-antibody antibodies [3]. Thus, there can be heterophilic antibody interference in immunoassays in patients receiving zu-mab, including during follow-up [5].

This class of antibodies includes Actemra[®], Avastin[®], Cimaher[®], Entyvio[®], Gazyvaro[®], Herceptin[®], Keytruda[®], Lemtrada[®], Lucentis[®], Mylotarg[®], and Perjeta[®].

2.4. Human antibodies (u-mab: with the “u” from “human”)

At present, u-mab is the final stage in obtaining therapeutic antibodies comprising only amino acid sequences found in human immunoglobulins (IgG). Various procedures have been described: combination from banks of human amino acids of variable and constant domains, or using transgenic mouse.

Administration should not induce antibodies targeting the therapeutic antibody, nor has this been so far reported. Moreover, any conceivable anti-human-antibody antibodies would lack affinity for the mouse antibody used as immunoassay reagent, and would thus not cause interference.

This class of antibodies includes Arzerra[®], Cyramza[®], Opdivo[®], Prolia[®], Vertibix[®], Xgeva[®], and Yervoy[®].

As can be seen in the lists presented above and in Table 1, the prefixes of origin (o, xi, zu or u) never figure in the names of the proprietary specialties, and the INN list has to be consulted each time to know which antibody class is concerned [6]. Year-by-year auditing of the FDA-approved therapeutic molecules for 1997 to 2014 [7] finds ever more zu-mabs and u-mabs on the market; however, this does not mean that older molecules, such as xi-mabs and o-mabs, are being abandoned.

3. Production of antibodies for immunoassay

3.1. Polyclonal antiserums

Immunoassays were invented in 1959 by Berson and Yalow [8] to assess insulin levels, using a reagent consisting of serum from a diabetic patient who had developed anti-insulin antibodies, therapeutic insulin at that time not being of human origin. Since then, antiserums have been obtained against all proteins, and against non-protein molecules such as corticosteroids if these are complexed with a protein. The resulting reagent is a mix of antibodies from different lymphocyte clones and is thus polyclonal; it can be purified to a greater or lesser degree to optimize affinity and specificity.

These antiserums are used for competitive immunoassays, with minimal antiserum concentrations to allow the analyte to compete with the tracer for antibody binding sites. As they are produced in living animals, quantities depend on the size and longevity of the animal: rabbit, goat or other relatively large mammal, but usually not mouse.

There is thus little risk of anti-mouse antiserum (HAMA) induced by o-mab, xi-mab or zu-mab, leading to troublesome interference in immunoassays using rabbit or goat polyclonal antibodies.

3.2. Monoclonal antibodies

As mentioned above, production of monoclonal antibodies was invented by Kohler and Milstein in 1975 and they quickly came to be used in immunoassays. The affinity of these antibodies, produced by hybrid cells, turns out to be almost always lower than that of polyclonal antibodies selected for immunoassay. Monoclonal antibodies are thus seldom used in competitive immunoassays, but more often in immunometry, where antibody levels are very high compared to the analyte, and especially in 2-site “sandwich” immunometry, in which the analyte is exposed to 2 different monoclonal antibodies each specific to a particular immunogenic site in the analyte. This requires the analyte to be large enough to avoid steric overload by the 2 antibody reagents: i.e., as large as a peptide comprising at least 10 amino acids.

Table 1
 Monoclonal antibodies used in oncology, by alphabetical order of proprietary name (syllable in red in the INN identifies the type of antigen, as explained in section 1).

Proprietary name	INN	Target	Indications	Market authorization
Actemra [®]	Tocilizumab	Interleukin-6	Castelman's disease	2009
Adcetris [®]	Brentuximab	CD30	Hodgkin lymphoma	2011
Arzerra [®]	Ofatumumab	CD20	Lymphoid leukemia	2009
Avastin [®]	Bevacizumab	Vascular Endothelium Growth Factor (VEGF)	Colorectal, ovarian, lung, kidney cancer glioblastoma	2004
Bexxar [®]	Tositumomab	CD20	Nhl	2003
Blincyto [®]	Blinatumomab	CD19 and CD3	Acute leukemia	2017
Cimaher [®]	Nimotuzumab	EGFR	Trunk glioma	2004
Cyramza [®]	Ramucirumab	VEGF receptor	Gastric cancer	2014
Entyvio [®]	Vedolizumab	Integrin alpha4beta7	Crohn's disease	2014
Erbix [®]	Cetuximab	EGFR	Colorectal, ent Cancer	2004
Flixabi [®]	Infliximab	TNFalpha	Crohn's disease	1998
Gazyvaro [®]	Obinutuzumab	CD20	Lymphoma	2013
Herceptin [®]	Trastuzumab	HER2	Breast cancer	1998
Inflectra [®]	Infliximab	TNFalpha	Crohn's disease	1998
Keytruda [®]	Pembrolizumab	PD1	Melanoma	2014
Lemtrada [®]	Alemtuzumab	CD52	Lymphoid leukemia	2013
Lucentis [®]	Ranibizumab	VEGF-A	Choroid neovascularization	2006
Mabthera [®]	Rituximab	CD20	Nhl, leukemia	1997
Mylotarg [®]	Gemtuzumab	CD33	Myeloid leukemia	2014
Orthoclone-Okt3 [®]	Muromomab	CD3	Kidney transplantation	1986
Opdivo [®]	Nivolumab	PD1-receptor	Melanoma, Bronchial cancer, Renal carcinoma, Lymphoma	2015
Perjeta [®]	Pertuzumab	HER2	Breast cancer	2012
Prolia [®]	Dénosumab	RankL	Bone metastases	2011
Remicade [®]	Infliximab	TNFalpha	Crohn's disease	1998
Removab [®]	Catumaxomab	EpCAM and CD3	Malignant ascitis	2009
Remsima [®]	Infliximab	TNFalpha	Crohn's disease	1998
Rituxan [®]	Rituximab	CD20	Nhl, leukemia	1997
Rixaton [®]	Rituximab	CD20	Nhl, leukemia	1997
Sylvant [®]	Siltuximab	Interleukin-6	Castelman's disease	2014
Truxima [®]	Rituximab	CD20	Nhl, leukemia	1997
Vectibix [®]	Panitumumab	EGFR	Colorectal cancer	2006
Xgeva [®]	Dénosumab	RankL	Bone metastases	2010
Yervoy [®]	Ipilimumab	CTLA4	Advanced melanoma	2011
Zevalin [®]	Ibritumomab	CD20	Lymphoma	2002

NHL: non-Hodgkin lymphoma.

As these monoclonal antibody reagents are almost always derived from mouse lymphocytes, there is always a risk of analytic interference in assays on samples taken from patients receiving a mab containing mouse sequences.

Recently, it became possible to select monoclonal antibodies with sufficient affinity to create a competitive immunoassay for a steroid hormone. This means that interference from heterophilic anti-mouse antibodies can be also expected in certain steroid assays for patients under immunotherapy, unless mass spectrometry is used for assaying.

Table 1 presents monoclonal antibodies currently authorized in oncology, in alphabetic order of proprietary name.

4. Anti-Mab antibody interferences in immunoassays

There is a risk of interference in patients who develop antibodies against the therapeutic antibody, and more precisely against the non-human sites on the antibody, in assays using murine antibody reagents: i.e., hormone assays using at least one monoclonal antibody. Depending on the case, the interfering antibody could be a HAMA (antibody against mouse antigens), HACA (against chimeric antigens) or HAHA (against humanized antigens) [3].

Reports of side-effects of monoclonal antibody treatment sometimes give the percentage of subjects developing such

antibodies; summarily, according to the above-cited reviews, the probability of finding antibodies against therapeutic mabs is around 90% for o-mab, 40% for xi-mab, 10% for zu-mab, and almost certainly 0% for u-mab. There are, however, no data for possible analytic interference, which can only be described in the light of what is known about immunoassay mechanisms.

Anti-mouse antibodies developed by patients basically lead to two types of interference:

- the induced antibody may attach to both reagent antibodies simultaneously, in a false sandwich; in that case, the signal will be high level, indicating a false high analyte level;
- alternatively, the amount in the sample may be such as to saturate all reagent antibody sites, making any sandwich impossible; in that case, the signal will be very weak, indicating a false low analyte level.

This type of interference can be neutralized by pretreatment with HAMA blockers, such as heterophilic blocking tubes, ahead of the immunoassay.

This does not in itself rule out other interferences unrelated to immunotherapy, such as with components of the assay signal (streptavidin, biotin, etc.), which can increase or decrease the result and may involve one or several endocrine parameters. They are often manufacturer-dependent, and can usually be unmasked by changing analytic systems.

Thus, in patients treated by a mab containing murine sequences, analytic interference may be suspected in case of either low or high levels on immunoassay, which entails that the appropriate reference values need to be available.

5. Reference values

5.1. Definition

By definition, reference values are derived from a well-selected sample of the “reference” population, notably taking account of age and gender. The reference interval should be calculated as percentile rather than by mean \pm standard deviation, as the distribution of individual values is rarely normal. The lower and upper limits are calculated as the 2.5 and 97.5 percentiles if the interval is to include 95% of supposedly normal subjects, or the 5 and 95 percentiles if 90% will do. It should also be borne in mind that each limit is subject to a confidence interval corresponding to uncertainty, the range of which depends on the size of the sample used. Briefly, that such and such a value is above or below “normal” can only be asserted with caution.

5.2. Reference intervals

For a given hormonal parameter, there are as yet no valid universal reference intervals for whatever immunoassay. Differences between the reagents on the market are often large, as can be seen from inter-laboratory quality control findings: ProBioQual in France, the College of American Pathologists (CAP) or the British National External Quality Assurance Scheme (NEQAS).

When using commercial reagents, it is thus necessary to abide by the manufacturer’s reference values, trusting that these are well-defined, or else by the laboratory’s own internal quality control data if any such are available.

6. Good-practice guidelines

Safe prescription of biological dosage based on immunoassay of samples from patients under monoclonal antibody treatment requires taking certain precautions, and communication between the prescribing endocrinologist and the corresponding biologist is essential in case of the slightest doubt:

- the result should be compared against the reference interval provided by the reagent manufacturer or established within the laboratory;
- in case of clinical-biological discrepancy, the antibody that has been administered has to be identified: murine (o), chimeric (xi), humanized (zu), or human (u); the probability of interference in an immunoassay using one or more murine antibody reagents ranges from virtually 100% for an o-mab to probably 0% for a u-mab;
- if the rate for the parameter is suspected to be artifactually low, it should be compared versus clinical findings; if need be, other secretory functions should be explored;
- if it is suspected to be artifactually high:
 - check parallelism by successive dilutions: absence of parallelism may indicate interference,
 - in case of discordance with the rest of the biological work-up and/or clinical findings, screen circulating blood for an antibody against the reagent antibodies, by precipitating (by means of polyethylene glycol) the immunoglobulins supposed to be present if dilution is possible, or neutralizing them on heterophilic blocking tubes for free hormones that cannot be diluted, and repeating the assay; the antibody detected at this step is not routinely identified, as this requires more sophisticated techniques, such as western blot.
- In case of discordance with the rest of the biological work-up and/or clinical findings, mass spectrometry assay should be used instead of immunoassay, to avoid issues of antibody reagents and interference.

Disclosure of interest

The authors declare that they have no competing interest.

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