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REVIEW ARTICLE

PHARMACOKINETICS OF BIO-THERAPEUTICS: Rituximab

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Abstract

Introduction:-

- The term Bio-therapeutics usually refers to therapeutic materials produced using biological means, including recombinant DNA technology.
- Therapeutic agents that are produced from living organisms or their products(including rDNA technology, biotechnological manufacturing & chemical synthesis using nucleotides or amino acid).
- Includes monoclonal antibodies antibody fragments, peptides, replacement factors, fusion proteins, oligonucleotides, and DNA preparation for gene therapy as well as vaccines.

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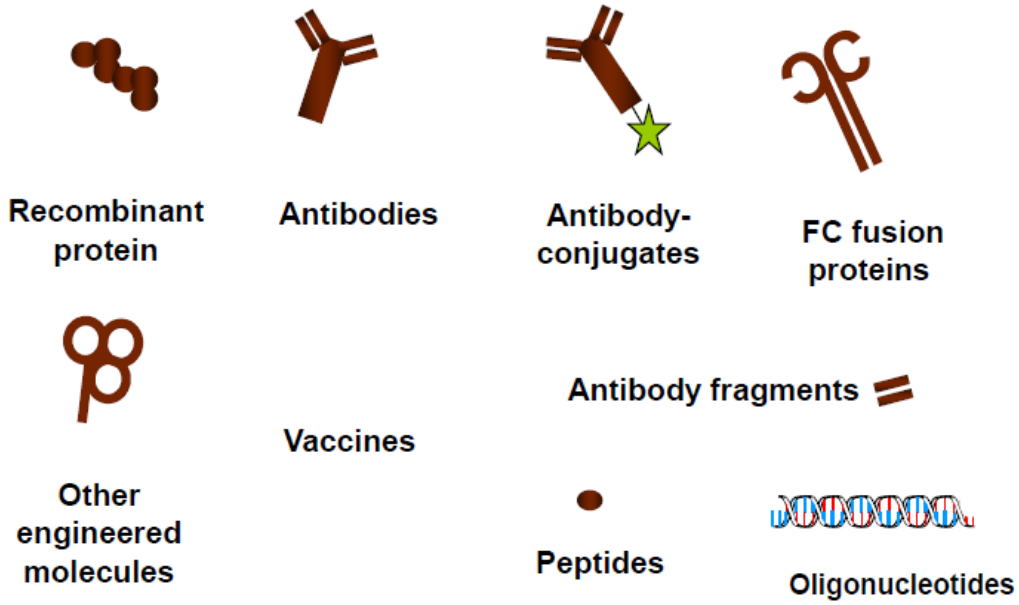
Types of Biopharmaceutical Modalities:-



How they are different from biopharmaceuticals:-

Biopharmaceuticals:-

- Small molecule drugs are organic or metallic compounds which bind with proteins in the body, thereby altering their function and their role in disease.
- Size is <600 Da
- Typically made utilizing chemistry synthesis.
- Work intracellularly.
- Less specificity.
- Easier to deliver (often oral, e.g., aspirin, antibiotics).
- Generally cheap to manufacture and easy to replicate after patent expiration (the high cost is the initial development)

Bio-therapeutics:-

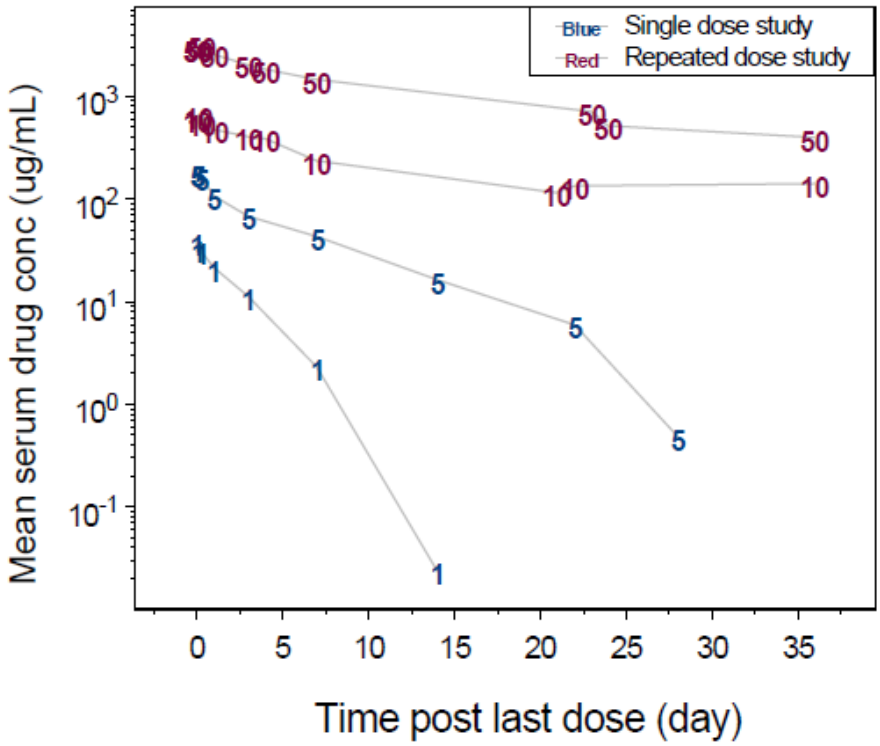
- Large molecule therapeutics treats diseases using biological matter, e.g., proteins monoclonal antibodies, peptides, RNA, cells, vaccines etc.
- Size is ~150,000 Da.
- Typically grown and extracted from living cells
- Work extracellularly.
- High specificity limits toxicities
- Difficult to deliver (usually must be injected)
- Generally expensive to manufacture.

Additional Challenges for Bio-therapeutics:-

- Delivery options are currently limited (main routes are intravenous and subcutaneous).
- Target Mediated Disposition may lead to **Nonlinearity.**

- Manufacturing is significantly more complex and a critical factor in safety and efficacy thus manufacturing changes have to be carefully assessed (**Comparability).**
- Products can lead to **immunogenicity where the body mounts** an immune response to the product. This is especially true for products that contain other species components (i.e. giving human protein to animals for safety studies).
- Species specificity may limit standard preclinical models for safety testing.
- First pass metabolism i.e g.i.t degradation.

Examples of Special Bio-therapeutics Considerations:-
• Nonlinearity



Comparability:-
Understanding potential changes in a product due to manufacturing changes.
• Comparable means "highly similar" not identical"
• ICH Q5E

"The demonstration of comparability does notnecessarily mean that the quality attributes of the pre-changed post-change products are identical; but thatthey are highly similar and that the existing knowledge is sufficiently predictive to ensure that any differences inequality attributes have no adverse impact upon safety and efficacy of the drug product".

Potential changes to manufacturing process:-
Expression system:-
Master cell bank
Working cell bank

Fermentation
Raw materials, cellculture conditions, scale,equipment, site change

Purification process:-
Column/resin, reagents,scale, site, equipment.

Formulation and filling:-
Excipient, liquid tolyophilized or *vice versa*,equipment, change in manufacturing protocol.

Drug product:-
– Batch definition, shelf-life,container/closure,shipping, storage.

Immunogenicity in Bio-therapeutic Development:-

- Immunogenicity refers to the production of an unwanted immune response directed at a bio-therapeutic.
- These are typically called anti-therapeutic antibodies (ATA) or anti-drug antibodies (ADA).
- Any exogenous protein may be viewed by the body as foreign and trigger immune response that lead to generation of endogenous antibodies against that protein.
- **Immunogenicity of biological products is a high profile concern for industry and for regulatory authorities**:
  - Immunogenicity may impact safety and efficacy.
  - FDA & EMA require that immunogenicity of bio-therapeutics be evaluated.

- Development of bio-therapeutics for chronic use is increasing the need to understand potential implications of immunogenicity.
- Immunogenicity strategies and data are essential components of Target Product Profiles, INDs, and BLAs.
- **Immunogenicity of the administration route: intradermal > inhalation > subcutaneous >intraperitoneal> intramuscular > intravenous.**

Clinical Impact	Clinical Outcome
Safety	➢ Hypersensitivity or anaphylactic reactions ➢ Neutralize activity of endogenous counterpart with unique function causing deficiency syndrome ➢ Immune complex formation
Efficacy	➢ Neutralize activity of therapeutic protein ➢ Increase or decrease efficacy by extending or curtailing half life ➢ Increase or decrease efficacy by changing bio-distribution
Pharmacokinetics	➢ Extend, or curtail half life ➢ Alter biodistribution ➢ PK changes may dictate changes in dosing

Advantages To Bio-therapeutics:-

- ❖ Favorable attrition rate.
- ❖ Minimum risk for non-mechanism based toxicity and safety issues.
- ❖ Can augment bodies normal growth factors, hormones, and enzymes.
- ❖ Able to modulate protein/protein interactions intractable to small molecules.
- ❖ Applicable in multiple therapeutic areas and for a variety of targets.
- ❖ Inherently highly specific for the target.
- ❖ Maintain natural biological activity.
- ❖ Broad range of protein expression capabilities,including enzymes,ctyokines,hormones and mAbs.
- ❖ Scalability.
- ❖ Safety,potency and consistency of the end product.

Pharmacokinetics Of Rituximab:-

- **Rituximab (RTX; Rituxan, MabThera)**is a **chimeric monoclonal antibody (mAb)** that binds the CD20 antigen, a transmembrane phosphoprotein specifically expressed by B-lymphocytes.RTX induces target cell death and is used in combination with polychemotherapy in the treatment of all histological types of B non-Hodgkin lymphoma (B-NHL) and in chronic lymphocytic leukemia (CLL), both as first-line and as rescue therapy. Furthermore, it is used for maintenance therapy of B-NHL and for treatment of several autoimmune diseases, in particular rheumatoid arthritis.

Doses and routes of administration:-

- RTX is usually administered **by intravenous (i.v.) injection**. The first approved schedule for induction therapy of B-NHL was 375 mg/m2 i.v. given for 4 cycles, and this was based on the pivotal trial of the antibody.
- Limited to the treatment of central nervous system (CNS) lymphomas, an intrathecal or intraventricular route has also been attempted since maximal RTX levels in cerebrospinal fluid are generally not more than 1% of serum levels after i.v. administration.Doses administered by the intrathecal or intraventricular route are generally 10 or 25 mg antibody every few days.
- A more convenient administration would be the **oral route,** but this is limited **by pre-systemic degradation** in the gastrointestinal tract, and by inefficient diffusion or convection through the intestinal epithelium. To bypass the low oral bioavailability, and as an alternative to i.v. administration, a number of mAbs are delivered **subcutaneously (s.c.).** The subcutaneous route has been employed for antibodies used in the treatment of allergy or autoimmune diseases, but more recently has been extended to trastuzumab, an anti-human epidermal growth factor receptor (HER)-2 antibody approved for treatment of breast cancer.

The proposed s.c. dose for RTX is a fixed dose of 1400 mg.

General aspects of RTX PK:-

- PK of RTX has been mostly studied after i.v. administration. In this case, RTX disposition is characterized by a 2-exponential decay, with a long elimination half-life of about 3 weeks (Fig. 1). The 2-compartment open PK model with first-order elimination represents the best structural model and seems to provide the best fit of RTX disposition, both during and after treatment, even with different schedules of drug administration.

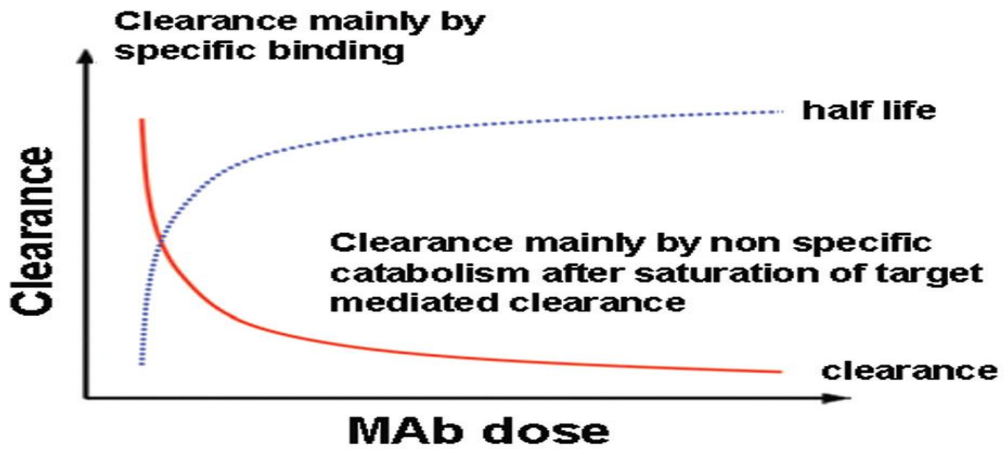


Figure 1:- Biphasic PKs of RTX. The model shows high clearance by specific binding to CD20) which, after saturation, leads to low clearance through non-specific binding via FcγR (RES).

Absorption:-

- After i.v. administration of RTX, all the drug administered reaches the systemic circulation (by definition the bioavailability (F) by this route is 100%), while after s.c. administration only a fraction of RTX dose (F ≅ 60%) is absorbed because, during the absorption phase, a portion of the drug undergoes proteolytic degradation or phagocytosis.
- Primary pathways for systemic absorption include **convective transport** of antibody through lymphatic vessels and into the blood, and **diffusion** of antibody across blood vessels distributed near the site of injection; however, on the basis of its molecular size, it is considered more likely that the RTX administered via s.c. injection is absorbed mainly via convection through lymphatic vessels.
- Generally, after s.c. injection, absorption occurs slowly and the time to reach maximum plasma concentration varies from 2 to 8 d. The bioavailability is determined by the extent to which the drug after s.c. administration undergoes pre-systemic catabolism and systemic absorption. In general, the absolute bioavailability reported varies from 50 to 100%.59 Clearly, RTX will also bind to CD20 on B cells after s.c.administration.
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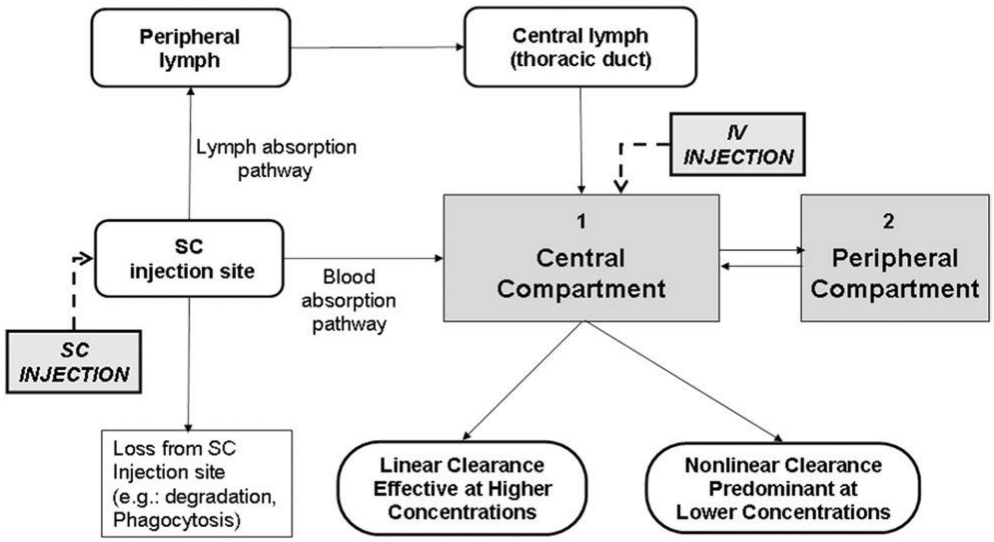

Figure 2:- Model of mAb absorption and clearance after i.v. or s.c. administration. Absorption and clearance pathways are shown.

Distribution:-

- Antibody distribution kinetics is influenced by rates of convective transport, binding to tissue sites, and rate of catabolism within tissue. After i.v. administration, RTX binds to the CD20 antigen present on the surface of normal or neoplasticB cells in the peripheral blood, bone marrow and lymph nodes.
- After distribution at the level of tissue blood vessels,There are different mechanisms of transport of the antibody from the systemic circulation through the capillary endothelial cells and into tissues. RTX diffusion across vascular endothelial cells is very slow and the movement of RTX through or between the cell membranes mainly occurs via transcellular (endocytosis) or paracellular mechanisms, i.e., convective transport of the antibody within the movement of the fluid flow
- For both i.v. and s.c. administration of antibodies, FcRn plays an important role by reducing mAb catabolism and mediating mAb transport across endothelial cells, thus promoting the distribution of the antibodies across tissues.
- The volume of distribution of RTX at steady-state is approximately 9.6 L. Since the plasma volume is only 3–3.5 L, this suggests that the mAb distributes into the extracellular spaces of tissues, except the CNS.

Elimination:-

- The total clearance is the sum of specific target mediated internalization, which is not linear and saturable, and non-specific clearance, which is linear and mediated by both FcγR-dependent and independent mechanisms (Fig. 1).
- Binding to FcRn generally reduces clearance because the antibody is recycled through FcRn to the surface and released into the cell environment. Therefore, FcRn binding protects mAbs from intracellular degradation.

The mechanisms of antibody eliminations are three:-

1. target-mediated elimination
2. proteolysis by the liver Kupffer cells and by monocytes/macrophages of the reticuloendothelial system (RES).
3. non-specific, FcγR-independent,endocytosis.

Accumulation:-

- Because RTX distribution and elimination are very long and clearance rate can vary, the extent to which the drug could accumulate after multiple doses is difficult to estimate. A steady-state condition (i.e., the condition in which, during each dosing interval, the intake of a drug is equal to the amount eliminated from the body) is achieved after approximately 3–5 half-lives**(Fig. 3).**
- In fact, small molecules **(e.g., chemotherapy)** have an elimination half-life in the magnitude of hours and rapidly achieve steady-state following administration(hours-days), while large molecules **(e.g., mAbs)** have a very long elimination half-life (in the magnitude of weeks) and may take up to 12 weeks to achieve steady-state.
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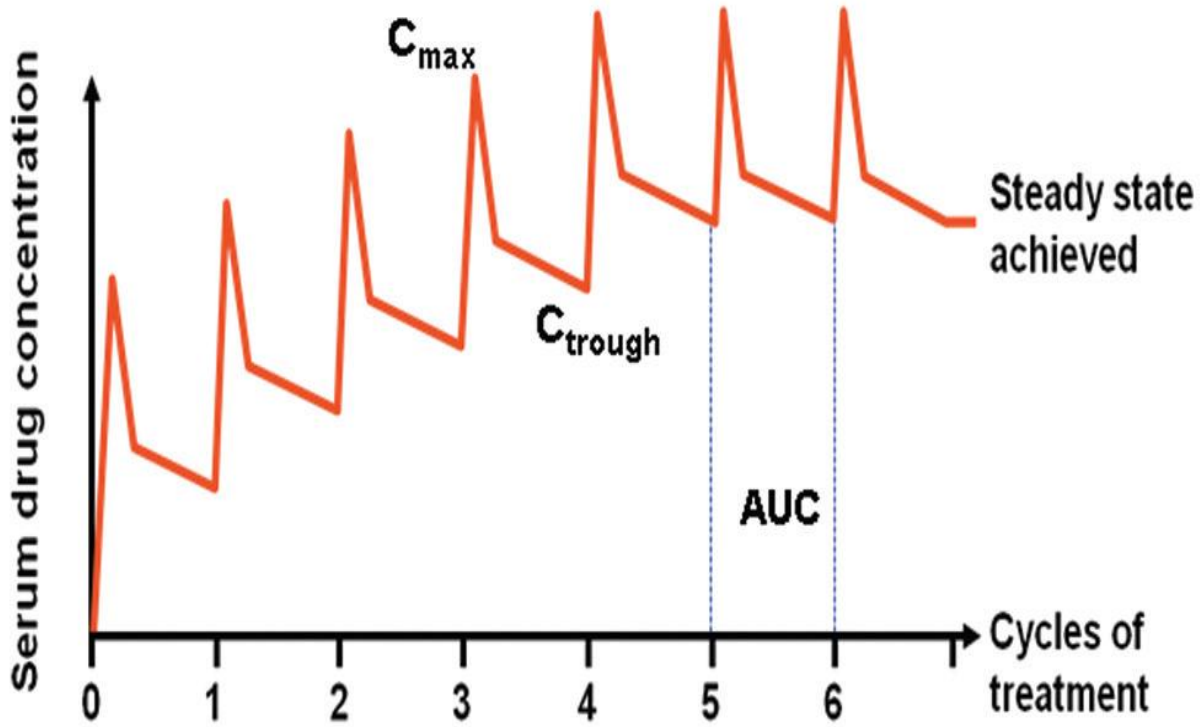

Figure 3:- Model of RTX PK after multiple dosing. Cmax, Ctrough, AUC and steady-state, reached after about 5 cycles, are shown.

Factors Affecting RTX PK:-

The association between tumor burden and RTX levels. Circulating RTX levels have been shown to be affected by the "tumour burden" (tumorvolume) in an inversely proportional way. Clinical studies have demonstrated that a high "tumor burden" is associated with low RTX serum levels. This is because thetumor cells act a sink for the antibody, adsorbing RTX through CD20 binding and inducing target-mediated elimination. A similar phenomenon is associated with the observation that a decrease in serum antibody levels during maintenance treatment can be predictive of relapse. Indeed, tumor growth adsorbs antibody, whose levels consequently fall in the circulation, in someCases anticipating clinical relapse.

Association between RTX levels and clinical response:-

Exposure to RTX (assessed by the area under the serum concentration-time curve [AUC]) and trough/pre-dose concentration of the drug are the PK parameters most frequentlyrelated to the patient's response (Fig3). Cmax represents a measureof drug concentration in the blood immediately followingi.v. administration, but it should be noted that during the firsthours after drug injection, the changes in serum concentrationdo not always reflect a proportional change in the concentrationof RTX in all other tissues, and hence in the amount of drug inthe body. The balance between plasma/serum and tissue concentrationsis obtained only a few days after drug administration.This is probably the reason why AUC and Ctrough are moredirectly related to clinical response compared

This has suggested that maintaining RTX above 25µg/ml would be beneficial.

Gender effects;-

An interesting aspect that has recently emerged is the effect of gender on RTX PK. Ctrough and AUC were generally higher in females than males both in the induction phase and in the maintenance phase, resulting ina better quality of response.

Comparison of Analytical Assays for Protein Quantitation:-

Variable	Immunoassay	Direct LC-MS	Immuno-capture LC-MS
Limitofquantification	5-200ng/ml	500-1000ng/ml	20ng/ml
Target Concentration	Mixture of free and bound	Total	Mixture of free and bound
Precision	Medium, No internal standard possible	High, with internal standard	Same as Direct LC-MS
Time for development.	8 months	Less than 1 month.	4 months
Throughput	High	High	Low,automation(robot)
Cost	Low	High	High

When to use LC/MS/MS

First Choice:-

- Peptides.
- Oligonucleotides in tissues.
- Early discovery (when no reagents are available).
- Antibody drug conjugates.

LC/MS/MS as a compliment to ligand binding assays (ex. ELISA)

• When there are issues with unresolved interference.
• Characterization – to understand what the ligand binding assay is measuring.

Conclusion:-

- ✓ Bio-therapeutics are becoming an increasingly important part of drugdevelopment.
- ✓ There are multiple types of bio-therapeutics each with their owndistinct characteristics and drug development challenges.
- ✓ Bio-therapeutics have unique challenges for drug development when compared to small molecules including (but not limited to) target mediated disposition, comparability, and immunogenicity.
- ✓ A key factor for Bio-therapeutics is the development of fit for purposebio analytical assays with the appropriate validations.
- ✓ Over the past 15 years, however, many data havebeen obtained on PK and PD and different prognostic factorshave been identified, allowing better prediction of what couldbe the best treatment regimens.
- ✓ At least in the context of B-cell malignancies, the maintenance of a minimum level of drug (currently defined as > 25 μg/ml) for a prolonged time (at least 200 days for induction therapy and up to 2 y for maintenance), seems to bemore important rather than the rapid achievement of a very highdose (200–300 μg/ml) for a shorter time.
- ✓ Better timing, simplified administration (fixed doses, s.c.) and different schedules with respect to chemotherapy are being introduced, on the basis of the present knowledge and of the widely different characteristics of therapeutic mAbs compared with standard drugs in terms of both PK and PD.
- ✓ Several of the lessons learned from RTX studies should be valuable for other anti-cancer mAbs.

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