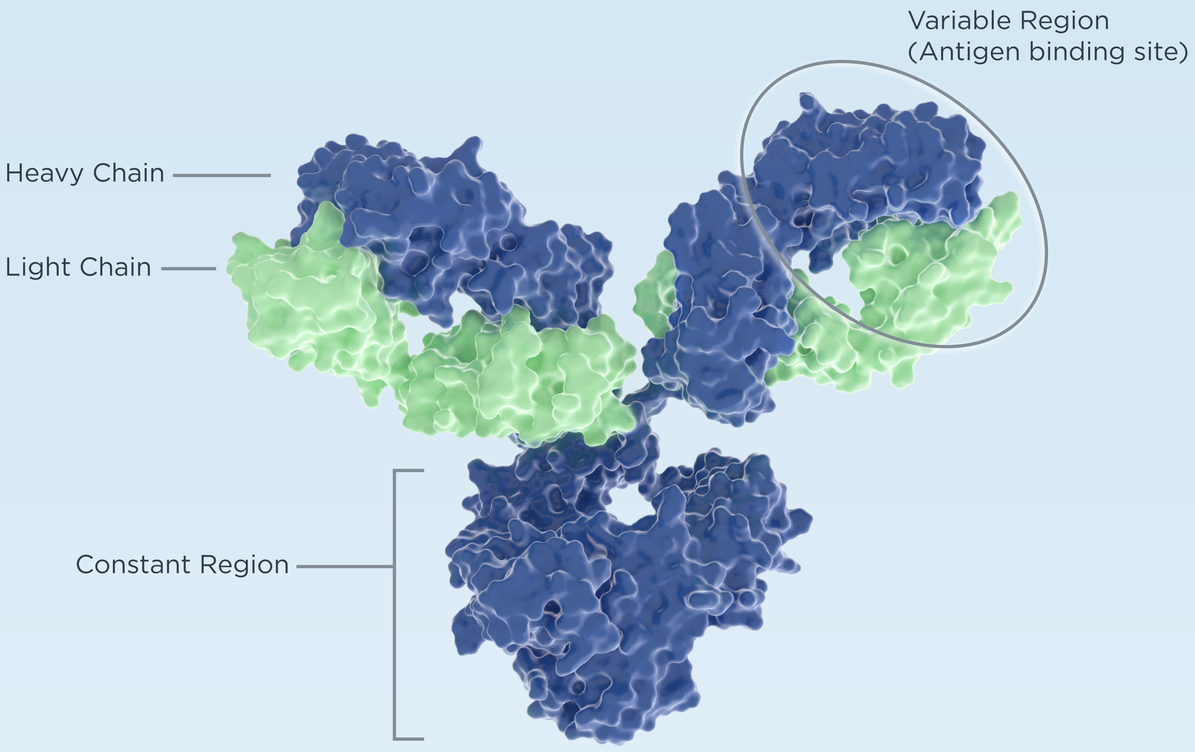
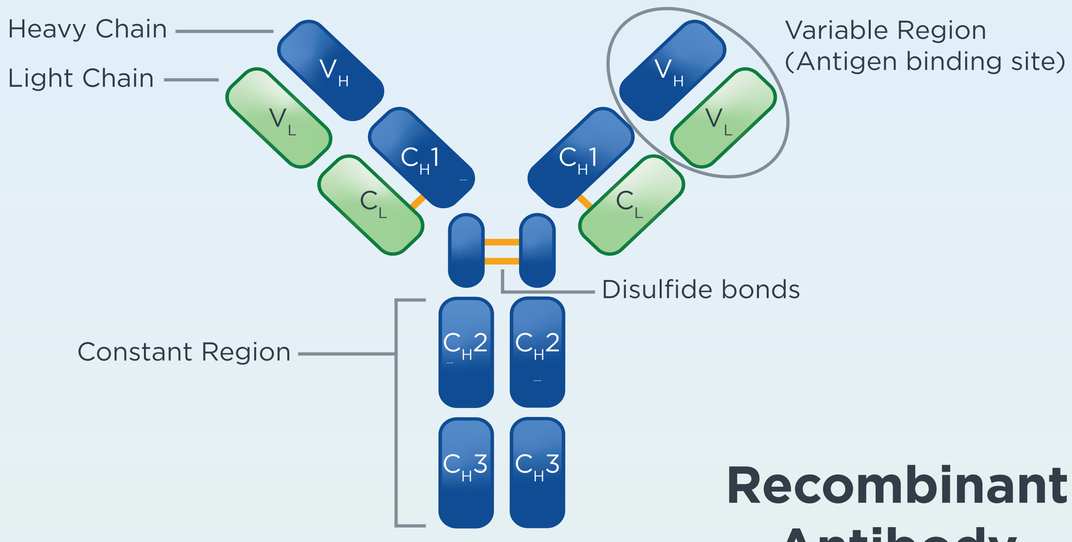
Encyclopedia of the Antibody

From Immunity to Humanity through Science

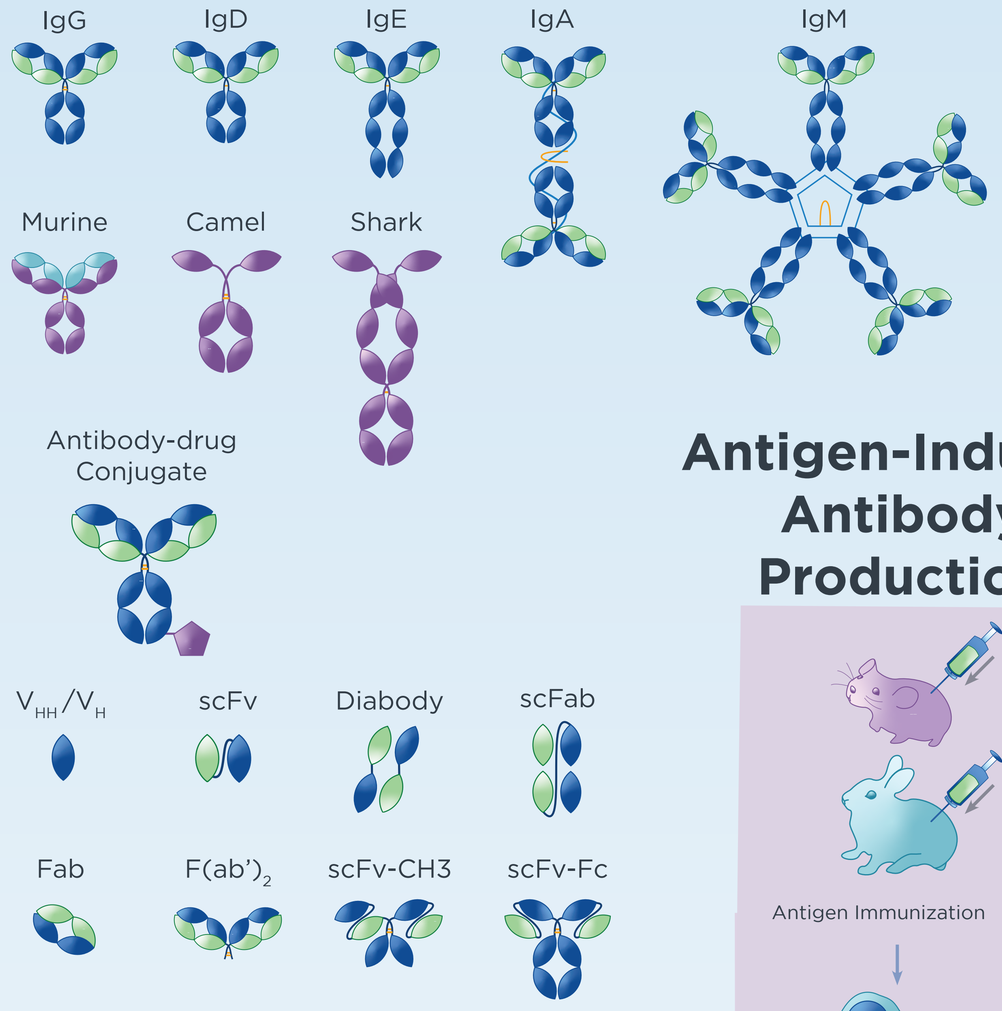
GenScript

Anatomy of an Antibody





Antibody Variations



Nomenclature

Atezolizumab

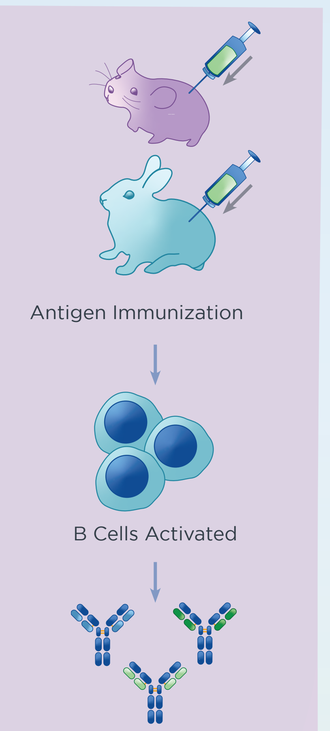
Prefix (unique variable name)

Target Substem
-ba-: bacterial
-ki-: interleukin
-li-: immunomodulating
-ta-: tumor

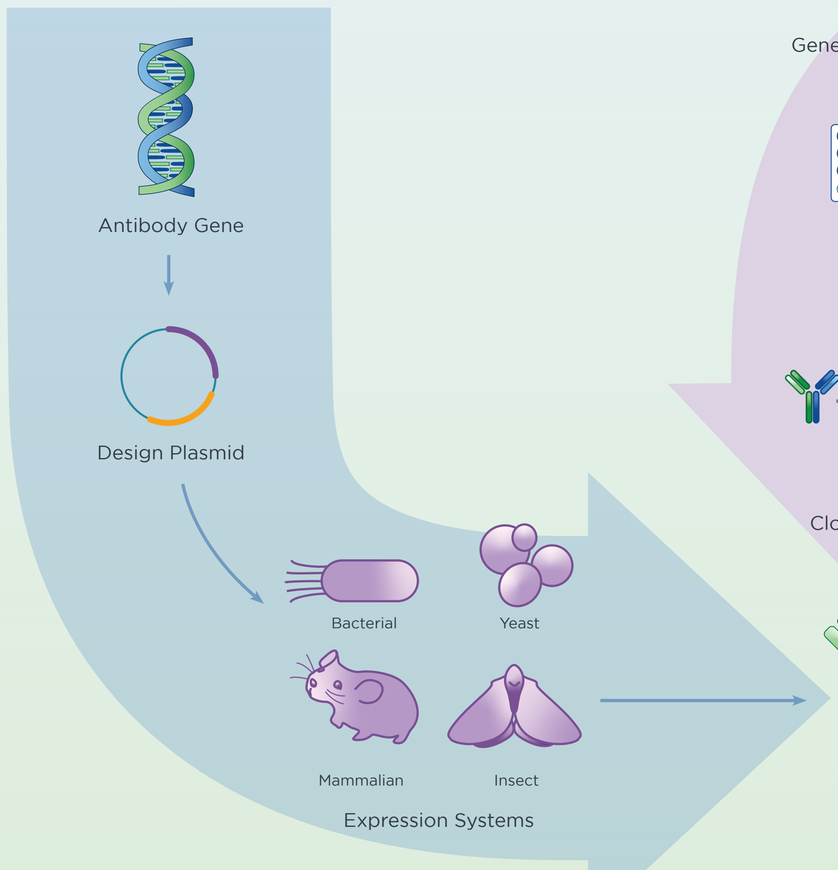
Source Substem *(discontinued in 2017)*
-a-: rat
-e-: hamster
-i-: primate
-u-: human
-zu-: humanized

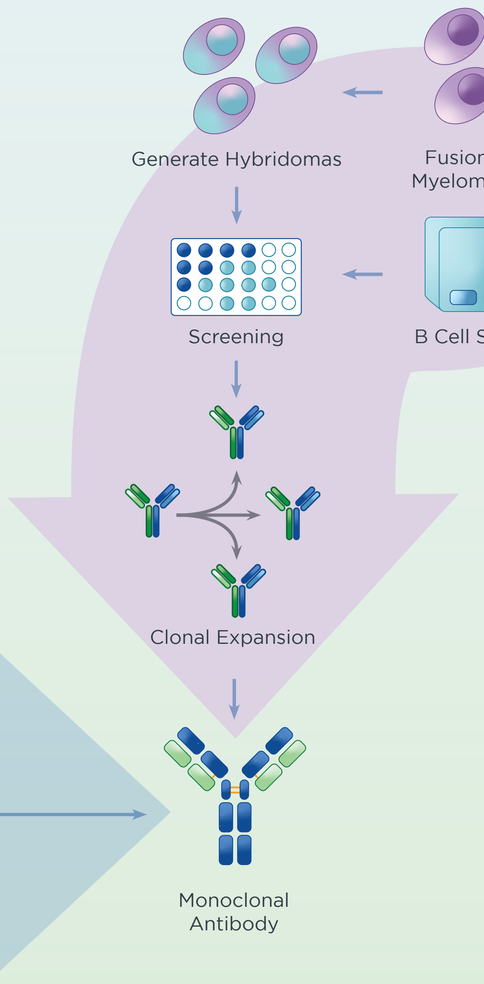
-mab indicates **M**onoclonal **A**nti**B**ody

Antigen-Induced Antibody Production



Recombinant Antibody Production





Monoclonal vs Polyclonal Antibodies

Monoclonal Antibodies

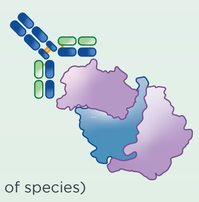
Definition: A monoclonal antibody refers to an antibody that normally recognizes only a single antigen (e.g. a single protein) and within which only a single common epitope is recognized.

Advantages:

- Constant & renewable supply
- Batch to batch consistency
- Homogeneity means more reproducibility
- Less background
- Highly Specific

Disadvantages:

- More expensive to produce
- Can be too specific (less likely to detect across a range of species)
- Cover only one epitope



Polyclonal Antibodies

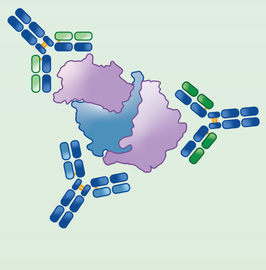
Definition: A polyclonal antibody refers to an antibody that normally recognizes only a single antigen but within which a number of different epitopes are recognized.

Advantages:

- Cheaper to produce
- Recognize multiple epitopes (robust detection)
- Higher tolerance for antigenic differences

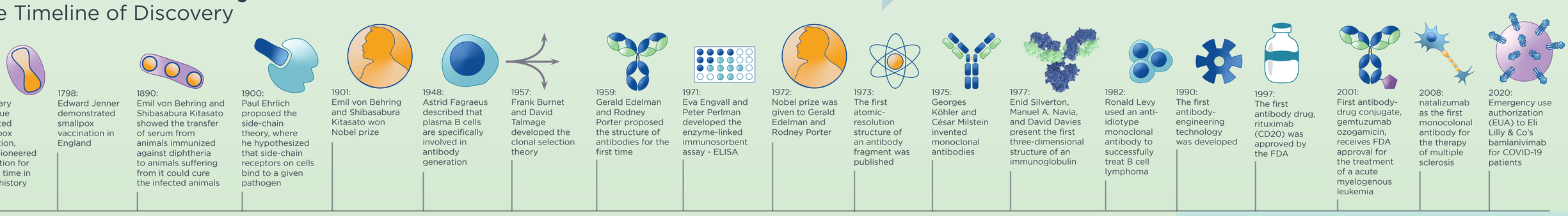
Disadvantages:

- Batch to batch variability
- High background
- Cross-reactivity due to multiple epitopes



Research History

The Timeline of Discovery



Glossary

ADC Antibody Drug Conjugates (ADCs): monoclonal antibodies (mAbs) attached to biologically-active drugs by chemical linkers with labile bonds. By combining the unique targeting of mAbs with the cancer-killing ability of cytotoxic drugs, ADCs allow sensitive discrimination between healthy and diseased tissue.

Affinity maturation: In immunology, affinity maturation is the process by which T_{cell}-activated B cells produce antibodies with increased affinity for antigens during the course of an immune response. In Ab drug discovery, affinity maturation is often applied to antibody leads selected from a naïve human library using a display technology. These leads may have relatively low (10–100 nM) target binding affinities but can be enhanced using various affinity maturation technologies to reach a desired affinity range (normally 0.1–10 nM).

Avidity: Refers to the accumulated strength of multiple affinities of individual non-covalent binding interactions, such as between a receptor and its ligand. It is commonly referred to as functional affinity.

Bispecific Ab: A bispecific monoclonal antibody (BsMAb, BsAb) is an artificial protein that is composed of fragments of two different monoclonal antibodies and consequently binds to two different types of antigen.

Camelid sdAb: Ab fragment consisting of single monomeric variable Ab domain that can selectively bind specific Ag.

CAR-T cell therapy: Chimeric Antigen Receptor-T cell therapy. A technique in which T cells are genetically engineered to produce special receptors on their surface called chimeric antigen receptors (CARs). CARs are proteins that allow the T cells to recognize a specific protein (antigen) on tumor cells. scFv fusion is normally used.

Complement-dependent cytotoxicity (CDC) assays: These test the efficacy of antibodies or protein-based drugs to activate a multi-pathway attack mediated by the complement immune system to kill specific target cells. The general method for CDC is to mix target cells bound by the antibody being evaluated with serum that contains the components of the complement system, often human serum, and then measure cell death.

Effector-function enhancement: The effector functions of an antibody refer to antibody-dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

ELISA: Enzyme-Linked Immunosorbent Assay.

Epitope binning: A competitive immunoassay used to characterize and then sort a library of mAbs against a target protein.

Epitope mapping: Epitope mapping is the process of experimentally identifying the binding sites, or 'epitopes', of antibodies on their target antigens. Identification and characterization of the binding sites of antibodies can aid in the discovery and development of new therapeutics, vaccines, and diagnostics.

Fab: The antigen-binding fragment (Fab) is a region on an antibody that binds to antigens. It is composed of one constant and one variable domain from both the heavy and the light chain.

FACS: Fluorescence-activated cell sorting (FACS) of live cells separates a population of cells into sub-populations based on fluorescent labeling.

Fc engineering: The Fc domain is critical to the functioning of an antibody and has been the focus of many engineering efforts. Broadly speaking, these approaches can be broken down into three main areas: increasing effector functions, decreasing effector functions, and extending serum half-life.

Fc fusion proteins: (also known as Fc chimeric fusion proteins, Fc-Ig, Ig-based chimeric fusion proteins and Fc-tag proteins) are composed of the Fc domain of IgG genetically linked to a peptide or protein of interest. Fc-Fusion proteins have become valuable reagents for in vivo and in vitro research.

Fc receptor assay: Used to measure Fc binding response using SPR technique. Assays are developed by covering the surface of a laboratory chip with recombinant human Fc receptors. Independently-prepared serum dilutions of test samples are then injected over two flow cells—a control cell and the Fc receptor–coupled cell—and the difference in binding response is then measured.

FcRn assay: SPR binding assay. The Neonatal Fc Receptor (FcRn) transports Igs across the placenta between mother and fetus. It is also responsible for salvaging internalized immunoglobulin and albumin, thus making it responsible for the long in vivo serum half-life of mAbs.

Humanization: Humanized antibodies are antibodies from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans. The process refers to the replacement of more than 90% of rodent IgG sequence in the parental antibody molecule with human IgG sequence.

Hybridoma stabilization: Antibody production by hybridoma cell lines is inherently unstable. Failure to maintain cell lines properly will lead to a loss in antibody productivity and eventually the cell line itself. Stabilization involves Ab sequencing, vector construction, and stable cell line generation for Ab production.

IC50: Half maximal inhibitory concentration. This is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. It is commonly used as a measure of antagonist drug potency in pharmacological research.

mAb sequencing: Knowing the sequence of a monoclonal antibody is not only the first step towards antibody engineering and function optimization, but also critical for patent application. The workflow involves: 1. mRNA isolation from hybridoma clone, 2. reverse transcription of mRNA to cDNA, 3. PCR amplification of H & L chain genes, 4. cloning into sequencing vector, 5. sequencing, 6. analysis.

Mechanistic study: A study or test designed to analyze the biological or chemical events responsible for, or associated with, an effect observed, and to provide information concerning the molecular, cellular, or physiological mechanisms by which substances exert their effects on living cells and organisms.

Monobody: Also known as Adnectins, monobodies are genetically engineered proteins that are able to bind to antigens. Despite their name, they are not parts of antibodies, which make them a type of antibody mimetic.

Monovalent Ab: Antibody with affinity for one epitope, antigen, or strain of microorganism.

Multivalent Ab: Antibody with multiple Ag binding sites. Most Abs are at least bivalent as they have at least 2 Ag-binding sites.

Naked mAb: Antibodies that work by themselves. There is no drug or radioactive material attached to them. These are the most common type of mAbs used to treat cancer.

Ortholog: Genes in different species that evolved from a common ancestral gene by speciation. Normally, orthologs retain the same function in the course of evolution. Identification of orthologs is critical for reliable prediction of gene function in newly sequenced genomes.

PD model: Pharmacodynamic model component into one set of mathematical expressions that allows the description of the time course of effect intensity in response to administration of a drug dose.

Phage display: Technique for the production and screening of novel proteins and polypeptides by inserting a gene fragment into a gene responsible for the surface protein of a bacteriophage. The new protein appears in the surface coating of the phage, in which it can be manipulated and tested for biological activity.

PK model: Pharmacokinetic model component into one set of mathematical expressions that allows the description of the time course of effect intensity in response to administration of a drug dose.

Reference antibody: Often used in in vivo proof-of-concept studies either to validate the target or establish an efficacy model. Can be a commercially available monoclonal antibody with a function similar to the intended therapeutic candidate, a polyclonal antibody functionally interacting with the target protein of interest, or an antibody reconstructed from sequences available in the public domain.

scFv: This is a fusion of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of 10-25 amino acids.

Species cross-reactivity: A desirable feature for a candidate antibody , refers to the ability of the antibody to bind and functionally interact with the orthologous proteins from various animal species used as models for evaluation of in vivo efficacy, pharmacokinetic and pharmacodynamic (PK/PD), and safety. The animals routinely used for these purposes include but are not limited to mice, rats, rabbits, and cynomolgus monkeys.

SPR: Surface Plasmon Resonance (SPR) is a technique that enables the detection of unlabelled interactants in real time.

Surrogate Ab: This is an antibody that is functionally equivalent to the therapeutic antibody candidate which binds specifically to the target ortholog expressed in the intended animal species.

Syngeneic model: Animal model used to test the efficacy of immuno-oncology antibody leads. Provides an effective approach for studying how cancer therapies perform in the presence of a functional immune system.

T issue distribution study: Conducted to evaluate PK in several species. Distribution of a drug between tissues is dependent on vascular permeability, regional blood flow, cardiac output, perfusion rate of tissue, and the ability of a drug to bind tissue.

Transgenic mouse model: A biological model that has been genetically modified by the introduction of a foreign DNA sequence/fragment into a mouse egg. The insertion of the foreign DNA usually results in a gain of function (expression of a new gene) or in the over-expression of endogenous genes.

Transient expression: Transiently transfected cells express the foreign gene but do not integrate it into their genome. Therefore, the new gene will not be replicated. These cells express the transiently transfected gene for a finite period of time, usually several days, after which the foreign gene is lost through cell division or other factors.

Xenograft model: Animal model typically used in cancer research. One of the most widely used models is the human tumor xenograft. In this model, human tumor cells are transplanted, either under the skin or into the organ type in which the tumor originated, into immunocompromised mice that do not reject human cells.

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