

# Immunotoxins

Immunotoxins are a new class of large molecules and targeted anticancer drugs. It is a conjugated protein containing an antibody, growth factor, or cytokine that binds directly to target cells and toxins (e.g. Diphtheria toxin) joined together with a pH-sensitive cross linker that serves as a disulfide bond in between. Like "magic bullets," it destroys cancer cells or virus-infected cells while having little or no effect on healthy cells.

## Components of Immunotoxins

### Antibody fragments

The hybridoma technique is used to create monoclonal antibodies. Antibodies have a large molecular mass, which limits their penetration into solid tumors with limited blood supply. A protease enzyme cleaves off the Fab portion of a monoclonal antibody to minimize its size. Currently single chain variable (scFv) fragments ( $V_L$  &  $V_H$ ) obtained by cloning into bacteria, which is a most favored method.  $V_L$  &  $V_H$  domains are then stably tethered together with flexible polypeptide linker. Cytokine and interleukins are produced by recombinant DNA technology, though plasmid transfer in E-coli bacteria.

### Antibody structure

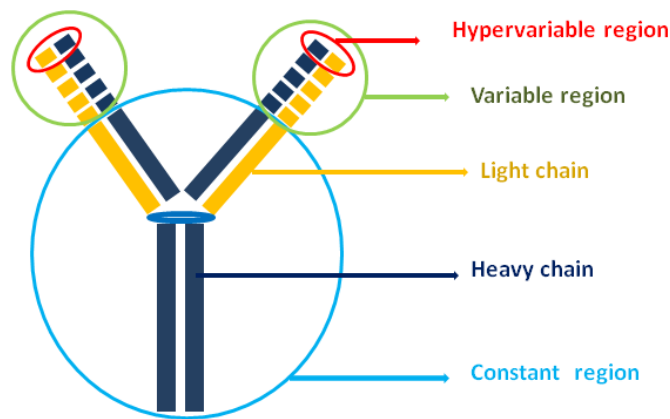


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## Toxins

Toxins are derived from bacteria, fungi and plants. Most commonly toxins are produced in *Escherichia coli* transformed with a plasmid encoding the recombinant toxin. They exhibit their function by inhibiting protein synthesis. Bacterial toxins kill cells by inhibiting the ribosome accessory protein called elongation factor-2 (EF-2) while plant originated toxins cleaves single adenosine from r-RNA.

Examples of toxins used in making Immunotoxins are:-

- **Bacterial toxins**

*Diphtheria toxin (DT)*

*Pseudomonas exotoxin (PE)*

*Anthrax toxin (AT)*

- **Plant toxins** – they are mostly ribosome inactivating proteins.

Ricin chain A (RTA)

Gelonin

Abrin

Pokeweed anti-viral protein

Dodecandron

## Linker

A cross linker connects the targeting moiety and the toxin. Internal disulfide bonds exist in the linker substance, which are stable extracellularly (in an oxidizing environment) but labile intracellularly (in reducing environment).

## Immunotoxin structure

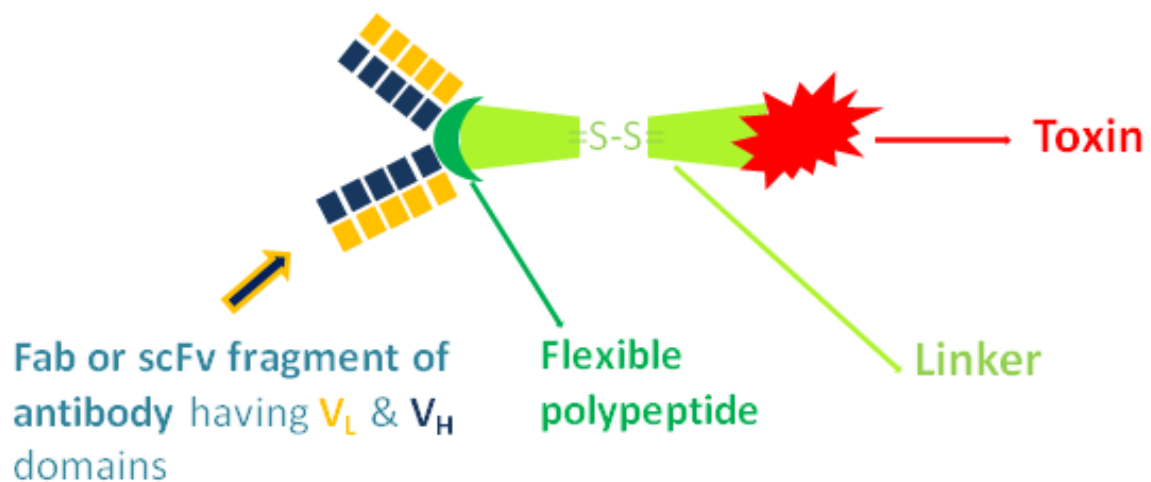


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## Mechanism of Immunotoxins

Immunotoxins float in the blood stream until they bind to their intended target cells. The antibody part (Fab) binds to the cell surface target receptor. Immunotoxin & receptor complex internalized by endocytosis, where it localized to an acidified endosome (eg. DT) or the endoplasmic reticulum or trans-golgi apparatus. In a reducing environment, the disulfide bond of the linker breaks. As a result, the linker that holds the toxin to the antibody is cleaved, and the free toxin exerts its cytotoxic effect by inhibiting protein synthesis of the cell.

## Mechanism of Immunotoxins

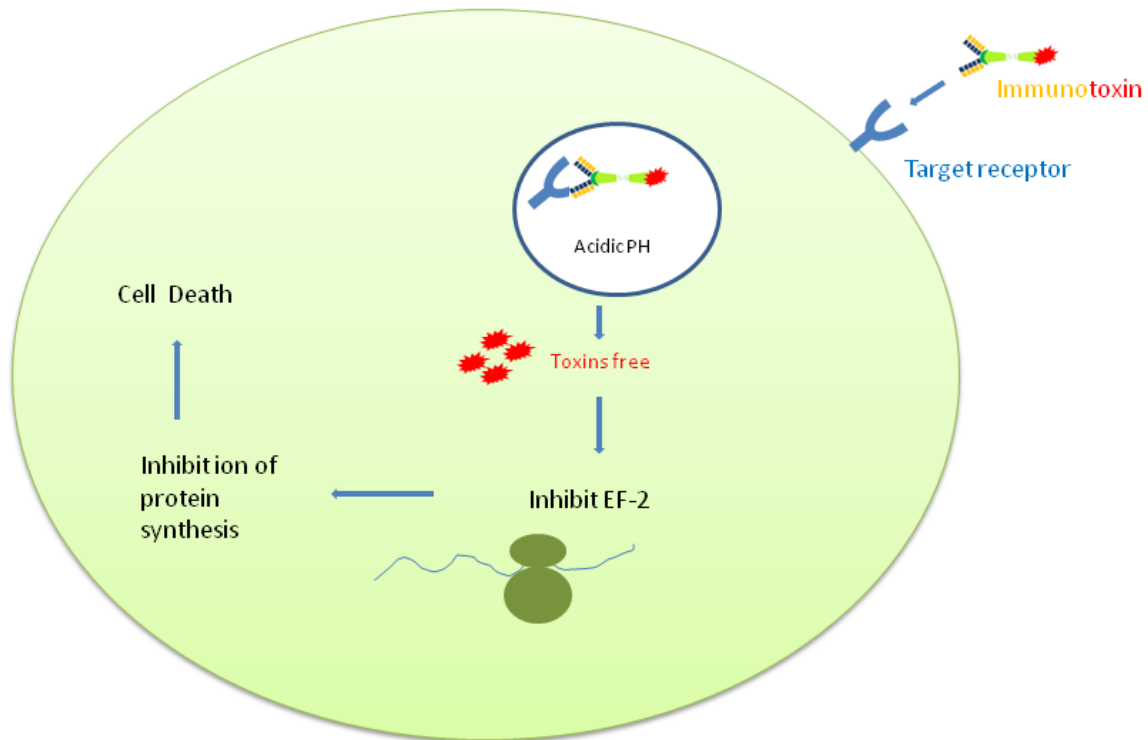


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## Uses of Immunotoxins

S.N.	Immunotoxin	Toxin	Antibody/GF	Uses	Status
1	LMB-2	Pseudomonas exotoxin (PE)	Murine monoclonal Ab against CD-22	HCL (Hairy cell leukemia)	Clinical development phase
2	BL-22 (Moxetumomab pasudotox)	Pseudomonas exotoxin (PE)	monoclonal Ab against CD-22	Lymphomas	US-FDA approved

<b>3</b>	Denileukin diftitox	Diphtheria toxin (DT)	Recombinant IL-2, which acts on CD-122 and CD-132	Cutaneous T-cell lymphoma	US-FDA approved
<b>4</b>	8H9-PE38	Pseudomonas exotoxin (PE)	monoclonal Ab 8H9	Neuroblastoma, RCC, childhood sarcoma, Brain tumor, breast carcinoma	Clinical development phase
<b>5</b>	RFT5-SMPT-dgA (Imtox-25)	Deglycosylated ricin-A chain (dgA)	RFT-5 murine monoclonal Ab	Hodgkin's disease	Clinical development phase

## Limitations of Immunotoxins

### Vascular leak syndrome

The carbohydrate residue of ricins, truncated Pseudomonas exotoxin, or IL-2 ligand can bind to endothelial cells in capillaries, causing cell lysis through a mechanism different from that of usual toxins.

### Renal toxicity

Recombinant Immunotoxins are excreted primarily through the kidneys, which can cause renal toxicity.

### Hepatotoxicity

Hepatotoxicity can result from the binding of basic monoclonal antibody residues to negatively charged hepatic cells.

### Immunogenicity

Immunotoxins, like all monoclonal antibodies, can trigger an immune response. Treatment failure may result from anti-Immunotoxin antibodies.

## **Production of Immunotoxin**

It is a time-consuming and resource-intensive process. Advanced technology and well-trained personnel are needed.