

Blocks In Apoptosis In Lymphoma & Drug Resistance

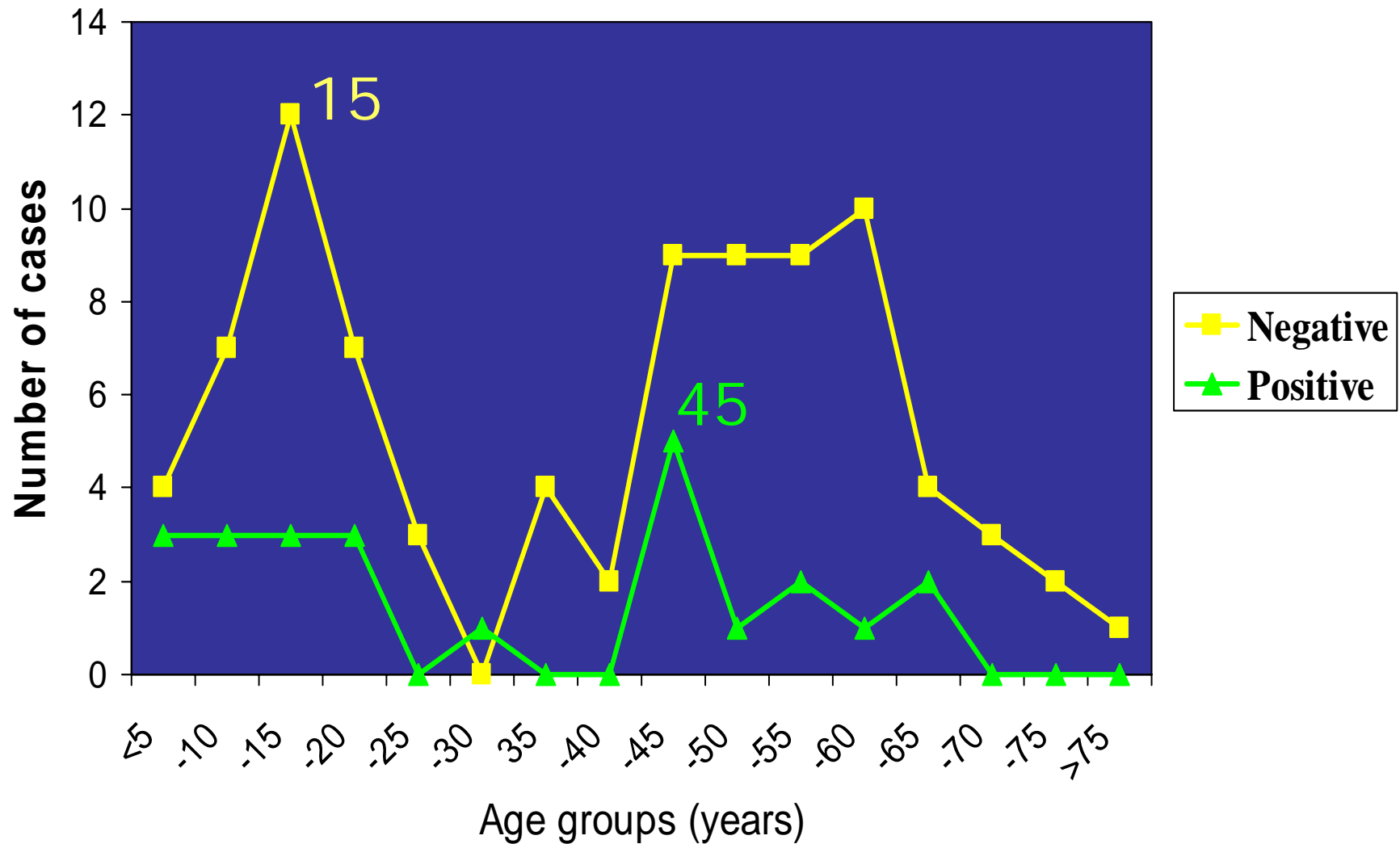
By

MAGDA ASSEM

**PROF. CLINICAL PATHOLOGY &
MOLECULAR BIOLOGY, NCI, CAIRO**

UNIVERSITY

Distribution of age by alk positivity In Egyptian Lymphoma



Blocks in Apoptosis in Lymphoma

- Molecular mechanisms & regulators of lymphocyte apoptosis.**
- Molecular aberrations that underlie drug resistance of lymphomas.**
- Potential therapeutic strategies that could circumvent these blocks in apoptosis in lymphoma.**

Apoptosis (PCD) plays an essential role in maintaining homeostasis by controlling cell number.

At the end of an immune response PCD delete lymphocytes with inoperative or autoreactive receptors to reverse clonal expansion.

CTL & NK induce apoptosis of target cells to cause innate & adaptive immune responses.

THE MOLECULAR MACHINERY OF CELL DEATH

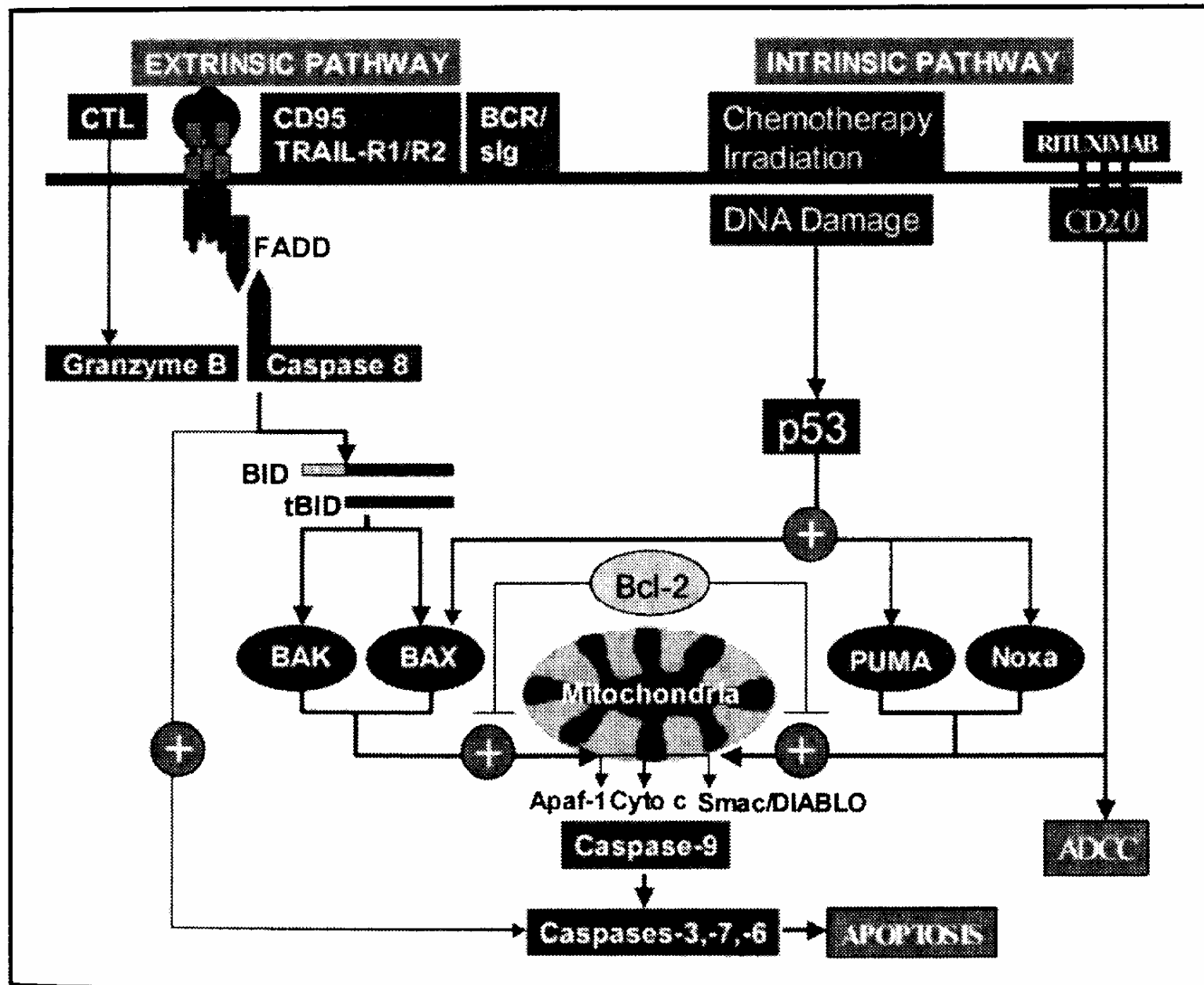
- **Caspases :cysteine aspartate proteases.**
- **Intracellular executioners of apoptosis.**
- **Recognize specific tetrapeptide motifs (in their target ptns) amino-terminal of the cleavage site, usually after aspartic acid residues.**
- **Activation is either by previously activated upstream caspases and/or by autocatalytic processing.**

THE MOLECULAR MACHINERY OF CELL DEATH

Involves activation of caspase family members by two major mechanisms:

- **INTRINSIC pathway.**
- **EXTRINSIC pathway.**

The intrinsic and extrinsic death-signaling pathways



Intrinsic Pathway

MOMP

Mitochondrial outer membrane

Permeabilization

**Signals the release of pro-death factors
(Apaf-1 & Cyto c) from the
mitochondria via the action of pro-
apoptotic members of the Bcl-2 family**

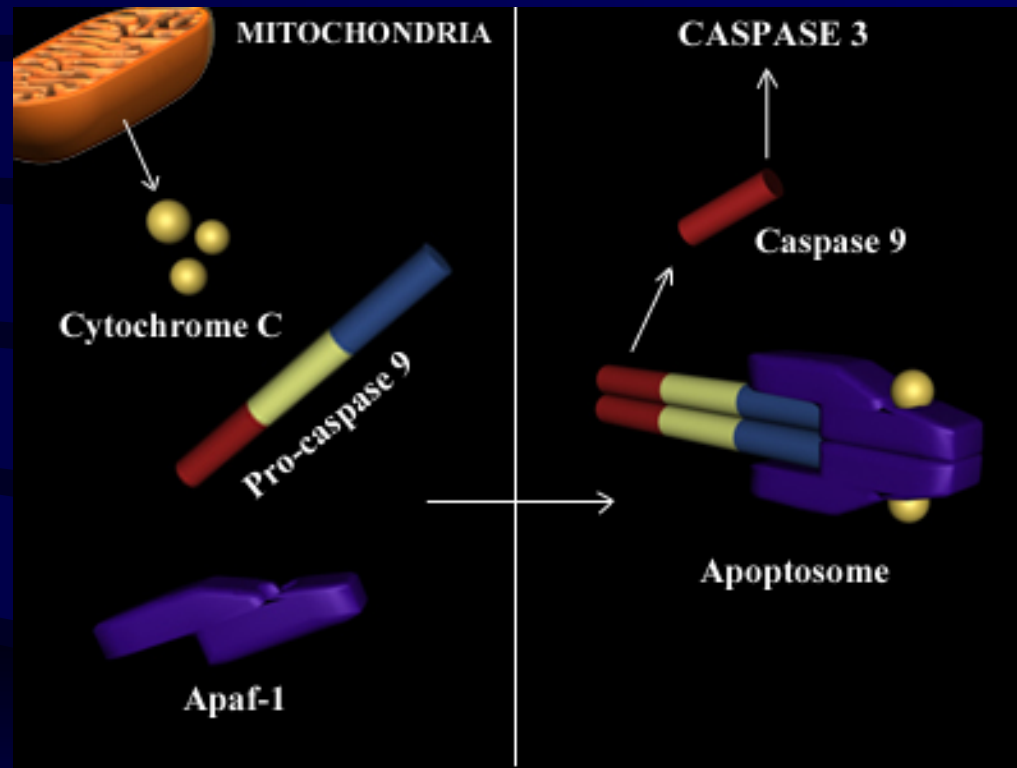
APOPTOSOME

Procaspase-9

Apaf-1 (apoptosis protease activating factor)

Cytochrome-C

Caspases



**The Formation of Apoptosome
(procaspase-9/ Apaf-1/cytochrome-c complex)**

Promotes transcatalytic cleavage

&

Scaffold-mediated transactivation of caspase-9

THE MOLECULAR MACHINERY OF CELL DEATH

Caspase-9 activates downstream caspases such as caspase-3 & -7, thus amplifying the caspase cascade & promoting apoptosis

However

The activity of caspase-9,-3,-7 is held in check by members of IAPs (inhibitor of apoptosis ptns)

**The final activation of caspases
–9,3 & 7
is mediated by the release of
two other ptns from the
mitochondrial intermembrane
space**

**Smac/DIABLO which binds &
antagonize the IAPs**

Extrinsic Pathway

triggered by activation of death receptors
(TNFR gene superfamily)

Death Receptors

TNFR1(p55or CD120a)

CD95(Fas or Apo1)

Avian AR1(DR3,Apo3)

TRAIL-R1 (DR4)

TRAIL-R2(DR5)

DR6

Ligands

TNF& lymphotoxin a

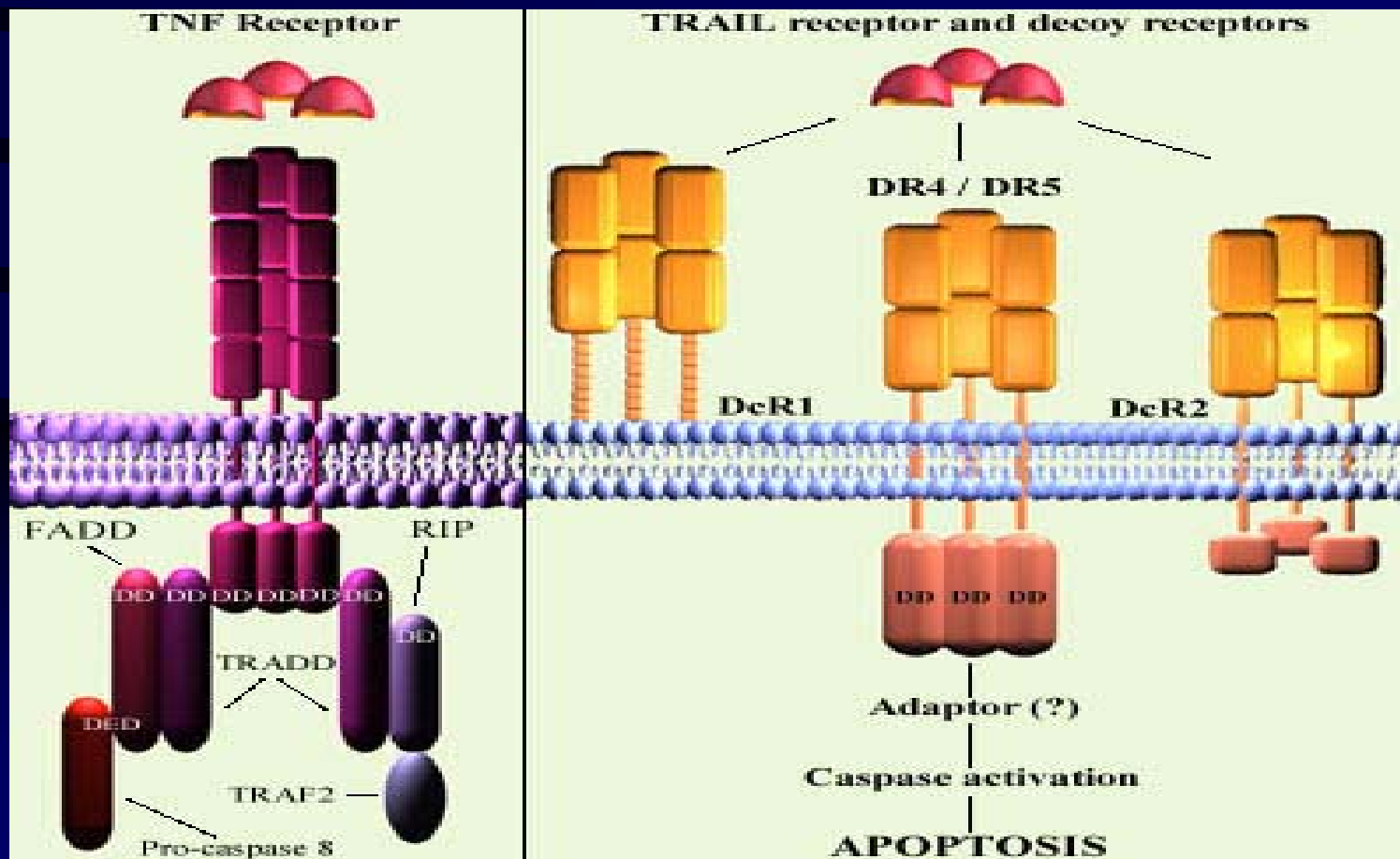
CD95L(FasLCtl,NkApc)

Apo3ligand(Apo3L)

Apo2L,TRAIL-R1

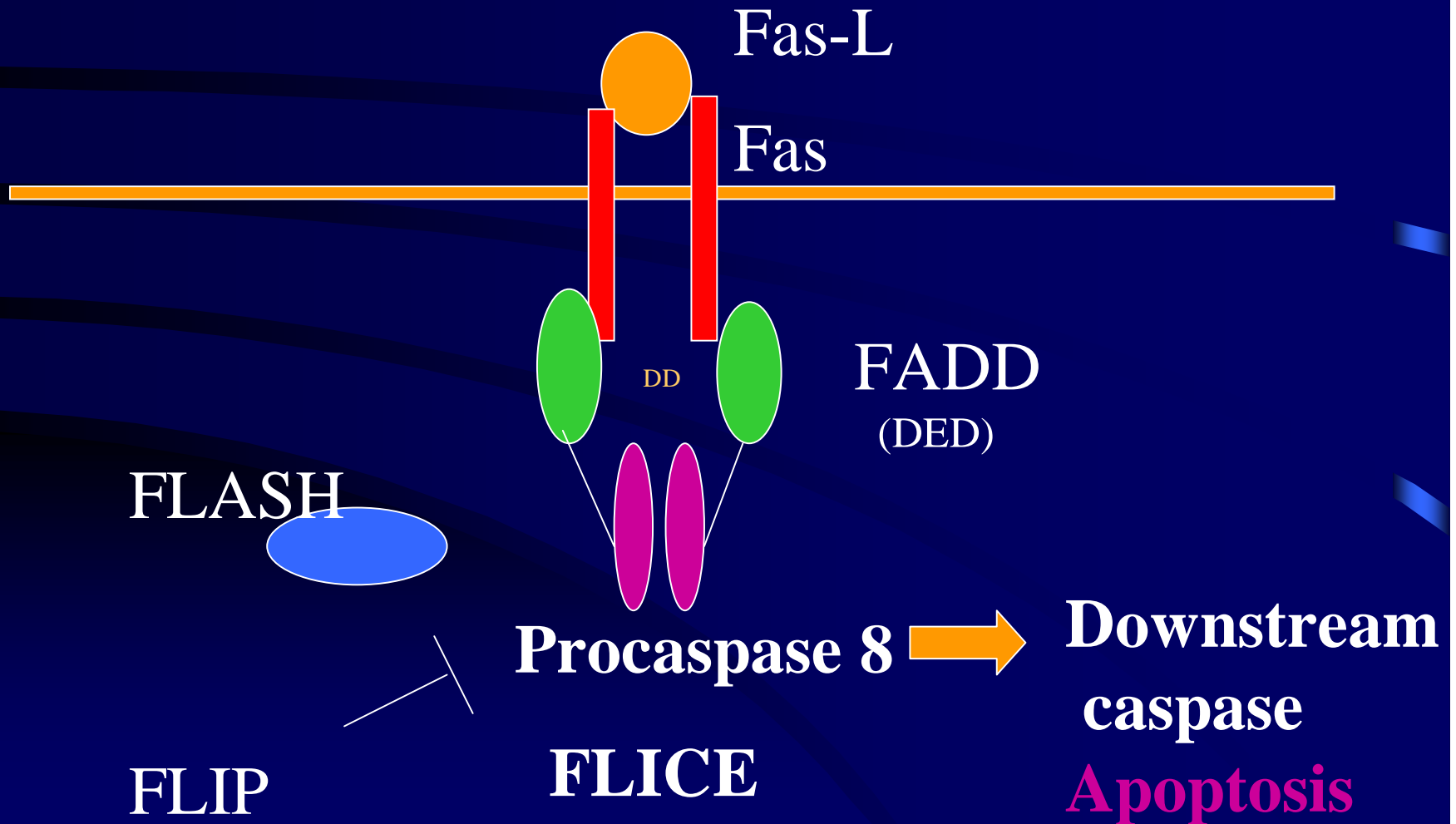
APO2L,TRAIL-R2

TNF Receptor Family



Death receptors are type I
transmembrane
cognate ligand engagement
death receptor oligomerization
assembly of DISC
adaptor ptn binding
FADD (Fas –ass. Death domain)
DED (death effector domain)
activation of caspases 8 & 10

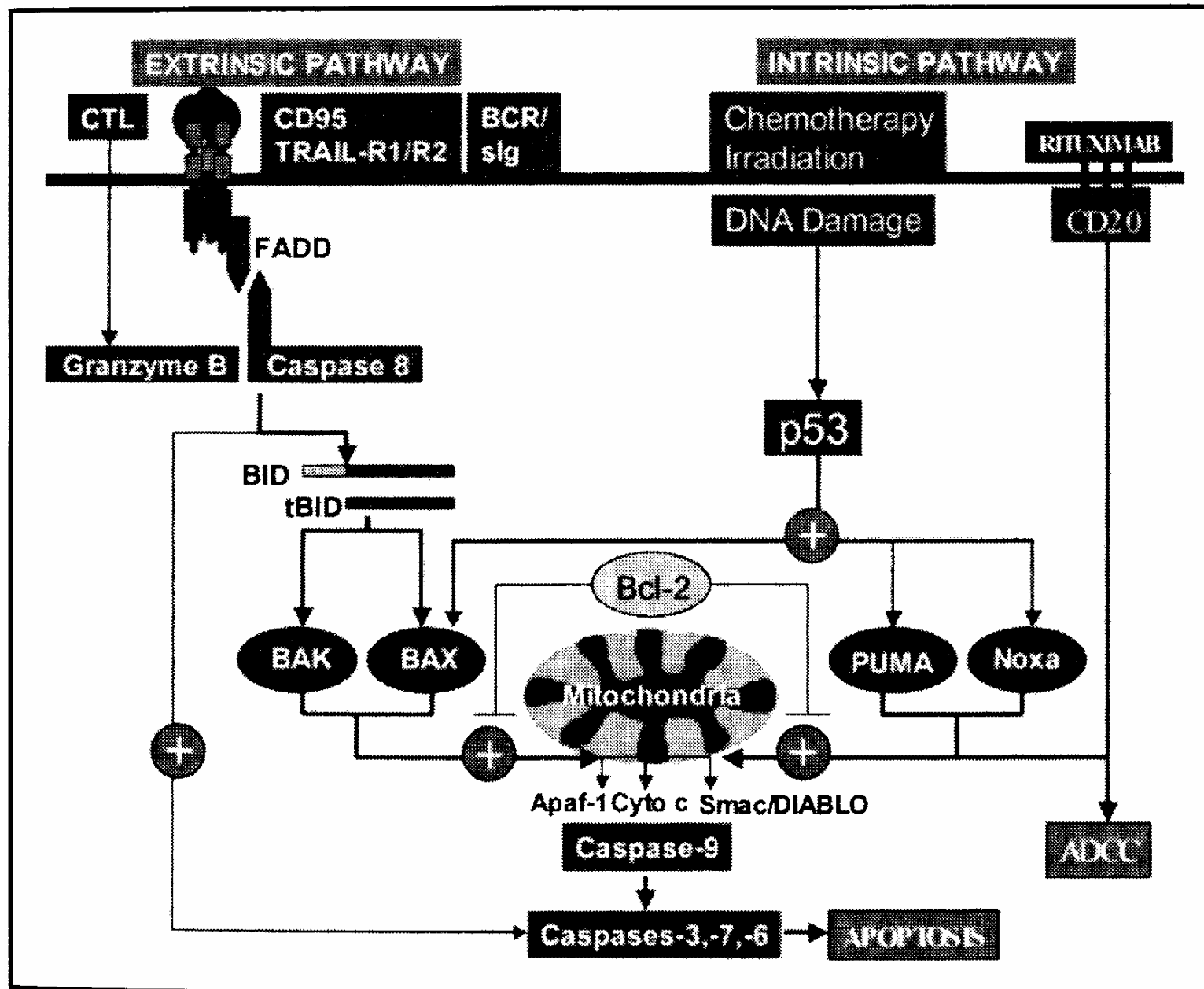
Death Ligand/Receptor Apoptosis



Is Execution of PCD similar?

- **Cell “type I”:**Activation of caspase-8 by the DISC (death-inducing signaling complex) results in direct activation of downstream effector caspase-3.
- **Cell “type II”:**Require MOMP for death receptor-induced apoptosis.

The intrinsic and extrinsic death-signaling pathways



Regulation of Death–Signaling Pathways

I-Regulation of MOMP by bcl-2 family.

II-Regulation of death receptor activation:

A-Expression of death & decoy receptors.

B-Regulation of caspase-8 by FLICE-inhibitory ptns.

C-Regulation of Bid cleavage (Casein Kinases I&II).

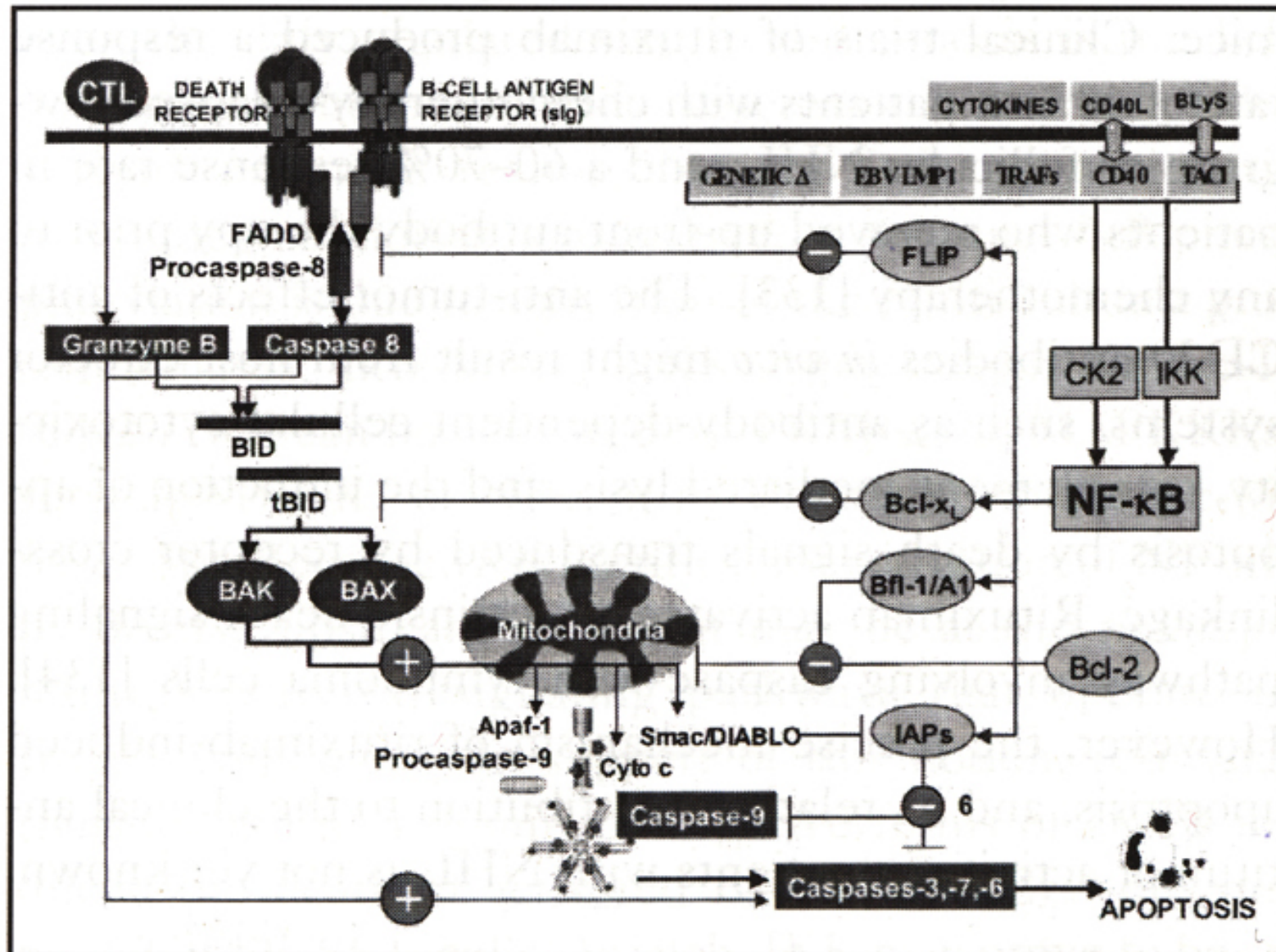
D-Sequestration of Bid by Bcl-x.

**III-Inhibition of caspases by inhibitor of apoptosis ptn
(IAP).**

IV-NF-kB.

**V-Dynamic balance bet death effectors &PCD
inhibitors**

Schematic representation of the molecular mechanisms and regulation of death receptor-induced apoptosis



V-Dynamic balance between Death effectors & PCD inhibitors

- **Inactivation of NF- κ B by caspase mediated proteolysis.**

Loss of survival gene expression.

- **Caspase mediated cleavage of Bcl-2, Bcl-xL & IAPs.**

Amplification of caspase activity.

Resistance of Lymphoma to Apoptosis

The ability of anticancer agents to induce tumor cell apoptosis is influenced by a host of oncogenes & tumor suppressor genes.

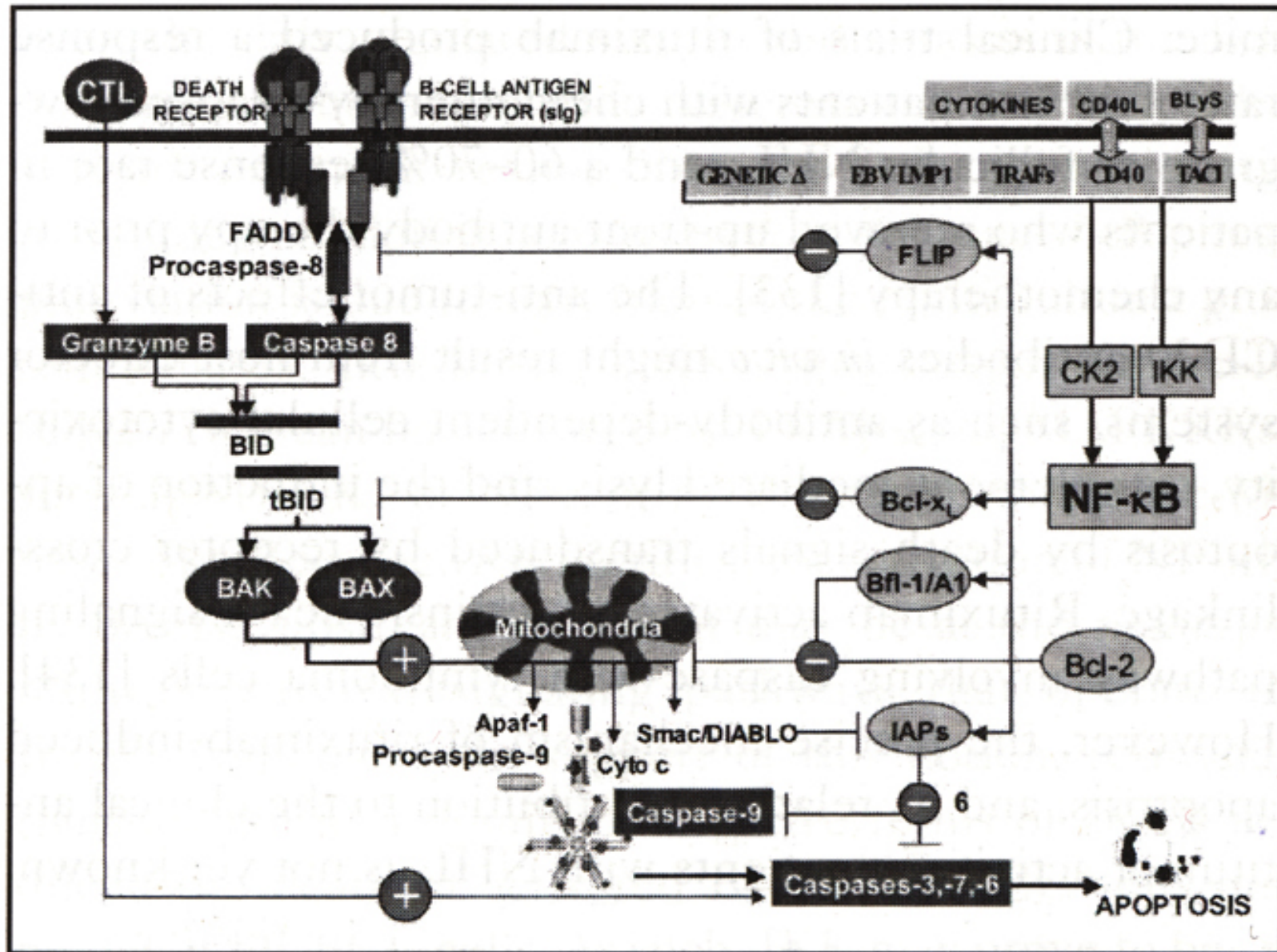
The effect of each antiapoptotic genetic lesion depends on where it interrupts a specific death-signaling pathway.

Lymphoma cells employ various mechanisms to evade apoptosis thus defying death

“RESISTANCE”.

Nature 2000,407:777-783.

Schematic representation of the molecular mechanisms and regulation of death receptor-induced apoptosis



Molecular Determinants of Resistance of Lymphomas to PCD

Three classes of antiapoptotic ptns have been identified in human B cell lymphomas:

I-Overexpression of Bcl-2,Bcl-xL:renders cells resistant to various ttt.

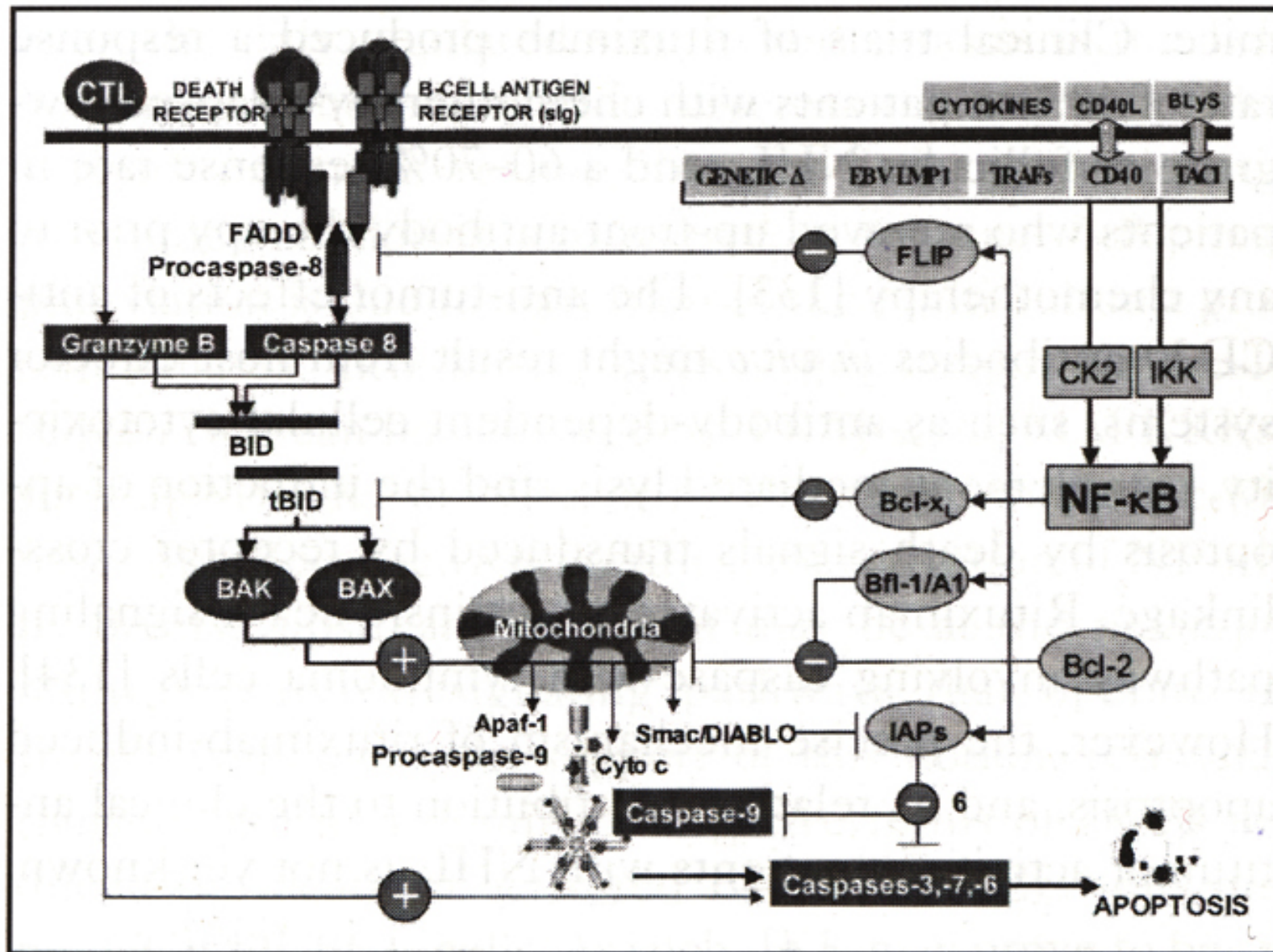
Overexpression of Bcl-xL:inhibits CD95L & Apo2L/TRAIL induced apoptosis (Cancer Res.2002,62:1583).

II-Survivin: IAP (50% High-grade NHL).

III-NF-kB exerts inhibition of cell death by:

++ survival genes:FLIP,IAPs,TRAFs&Bcl-xL, as well as repression of pro-apoptotic genes (Bax,p53).

Schematic representation of the molecular mechanisms and regulation of death receptor-induced apoptosis



Mechanisms to induce PCD in Lymphomas

How to overcome these genetic impediments to apoptosis?

I-Activation of alternate death-signaling pathways:

Anti-idiotypic vaccine.

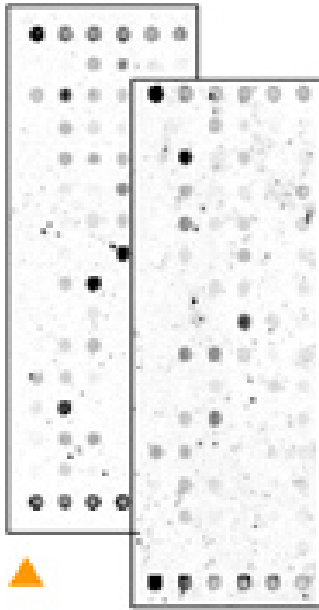
Anti-CD20.

CTL.

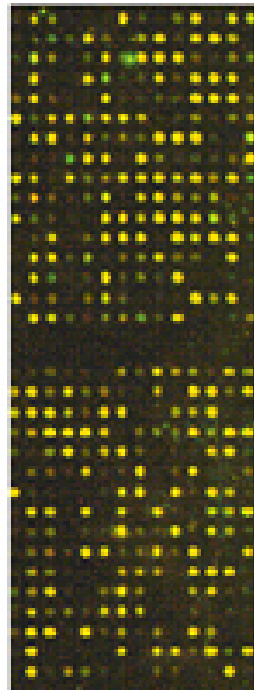
Apo2L/TRAIL.

II-Inhibition of key determinants of lymphoma cell survival eg bcl-2 , bcl-xL or NF-kB.

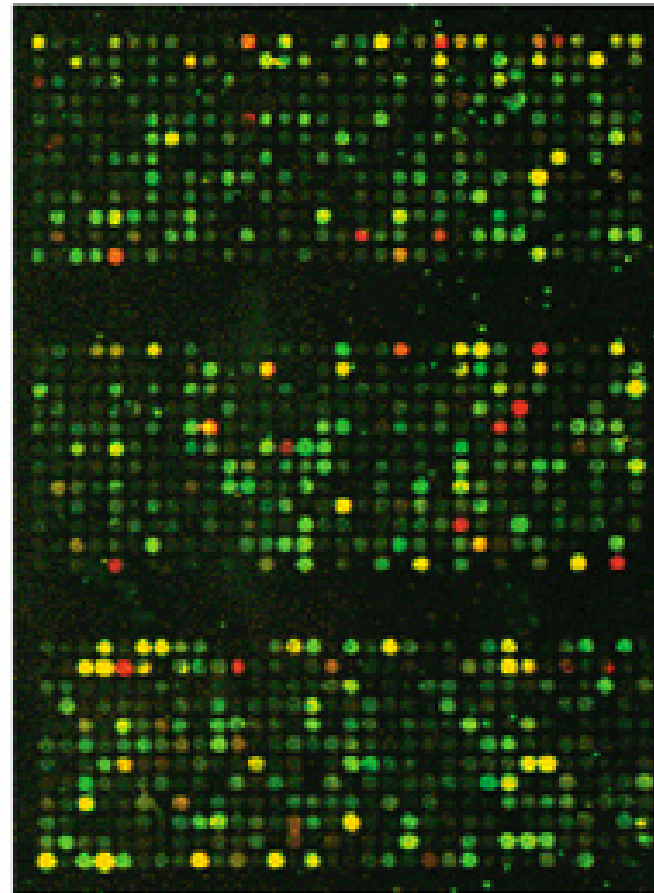
III-Combination.



▲ 1997



▲ 1998



▲ Today