* Tandom Mass Spectrometry *
(MS/MS) (MS^n)

→ Poinaple :- Based on the use of ≥ 2 MS arranged sequentially in a tandom ⊂ a collision cell placed b/w 2 mass filters.

Soft Ionization ↖

(Fast Ions)

1st MS is used to select a precursor ions of a particular m/z

↓

It is directed into a collision cell

↓

sample → Ionization source (Soft Ionization) → MS 1 → collision cell (Hard Ionization) → Product Ion → MS → 2nd or.

↓ Precursor ions

Ions collide ⊂ background gas molecules broken into smaller ions

↓

K/a "Product ions"

↓

Hard Ionization 2nd Mass filter, analyze the mass spectrum of product ions (daughter ions)

→ MF1 is scanned through the spectrum of precursor ions, while MF-2 is fixed to select specific product ion

↓

So ~~Scan of~~ MF2 ~~MF-1~~ Scan tells us ⊂ precursor ion produces a specific product ion

↓

Useful to analyze specific classes of analytes

ex:- Acylcarnitine measurement

↓

MF1 is scanned through the spectrum of precursor ions

↓

collision cell

↓

Product Ion formation

↓

The MF-2 is fixed for m/z - 85 Product Ion = ion acylcarnitine

Scan mode

A
B
C

gas → A+x, B+x, C+x

Static mode

→ x.

precursor ion scan

MS_1 — first MS scanned through range of m/z value

MS_2 — fixed to monitor m/z corresponding to x ionic species

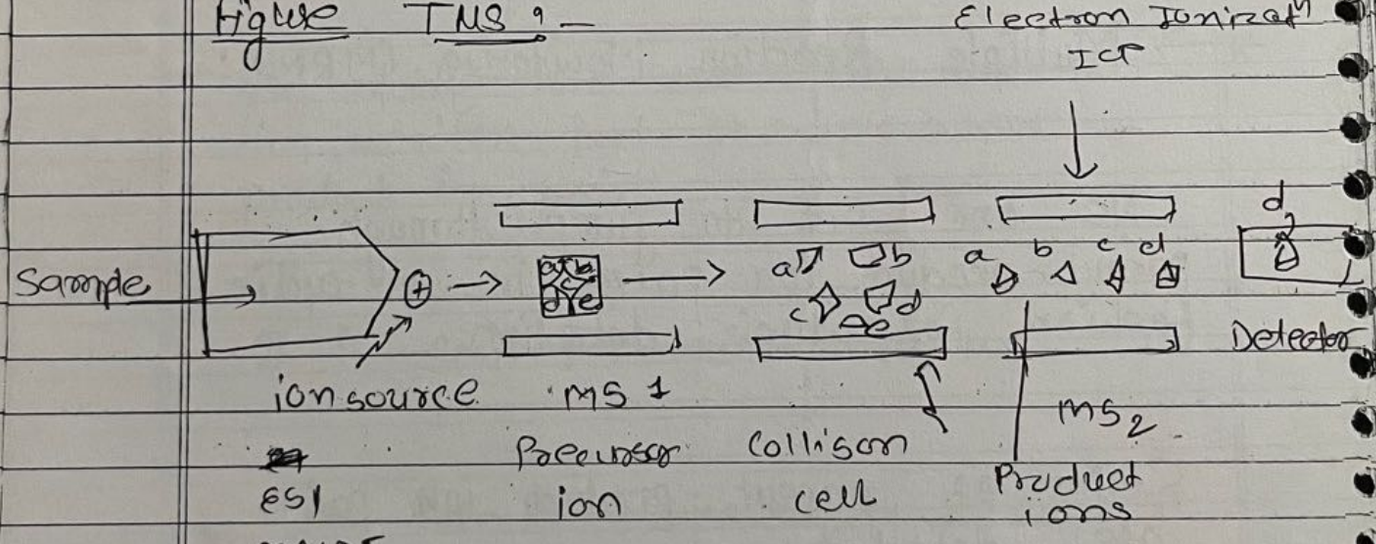
These 85 product ion only selected by MF_2 ↓

This will detected by detector.

→ Some analyte which produces 85 product ion — can be falsely detected in this → means can interfere in this detection.

* ~~Cons~~

Figure TMS :—



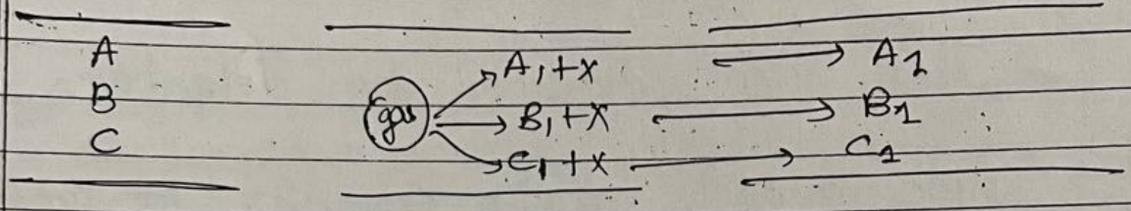
* Constant Neutral loss scan :—

→ two Mass filtes can scan synchronously ~~c~~ constant m/z offset b/w precursor & product ion

↓

This can indicates a ions less particular neutral fragment

eg :-



→ For A.A. quantification

* → Multiple Reaction Monitoring (MRM) :—

↓

Ms are set to jump through parent-product ion pairs in a cyclic fashion for their detection

↓

Series of parent-product ion pairs are detected.

↓

Useful for quantitative analysis of few selected target compounds.

eg:- if parent ion is of m/z 300 & product ion is of m/z 250 selected, then 300-250 pair

eg:- If MF 1 (Parent ion) = 300 m/z
MF$_2$ (product ion) = 250 m/z

↓

This is one precoursor - product ion pair

↓

MRM set to detect so many such diff. pair in a cydic fashion.

(MRM)

→ It can be set in a static mode

↓

In which two mass filter are set to monitor just one precoursor - product ion pair

↓

It is good for quantitative MS.

→ TMS detects compound by 2 physical properties precoursor ion mass & product ion mass

→ If it is combine with chromatographic seperation, 3rd physical property - retention time is added.

→ It gives very high degree of selectivity to the analysis & eliminate majority of potential interference.

→ TMS are also categorized as

Beam type Trapping type

→ Most popular type is tripple Quadrupole Beam type

→ Pressure is raised in Q-2 to the point that ions traversing Q-2 undergo several collisions

↓

dissociation of precursor ions

↓

formation of product ions.

* Magnetic Sector MS :-

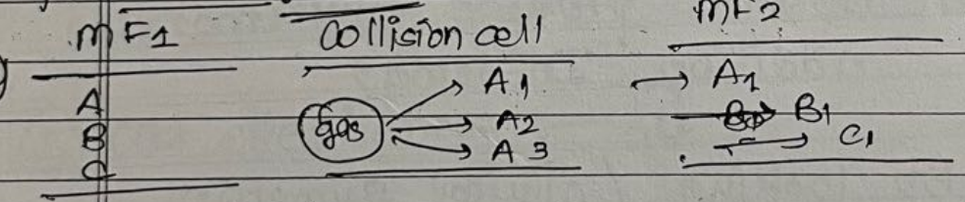
→ 2 magnetic sector instrument operated in tandem

* Hybrid MS :-

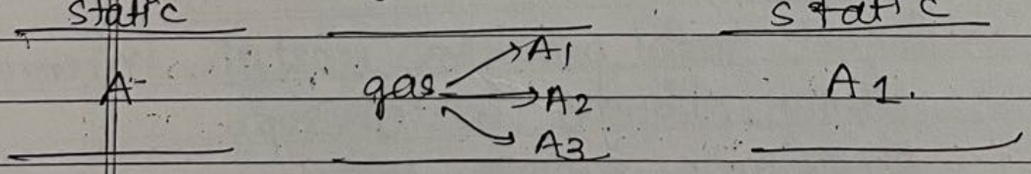
→ This include a combination of 2 different types of MS. in a tandom arrangement.

eg:- Quadrupole for the MF_1 & TOF for MF_2

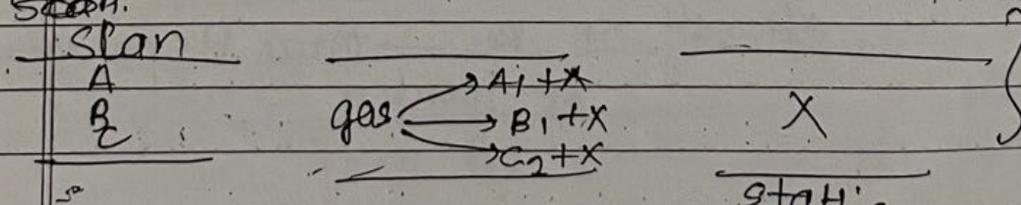
* ~~mrm~~ MRM :-



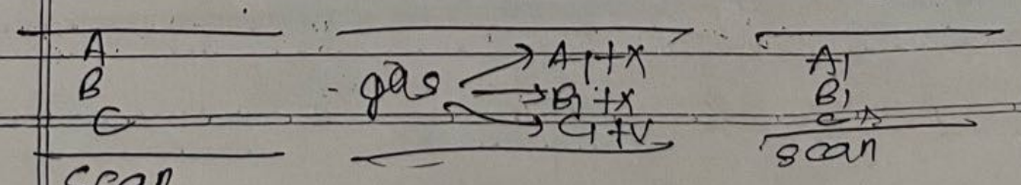
(B) mrm of Single compound. ~~(static)~~



(a) ~~Scan~~ Scan



(b)



Hieschy of evidence

(1) Technical performance — more precise & accurate
↳ Test method X is Better than Y
eg PSA vs PAP
OCPC vs Arsenazo ↓

(2) Diagnostic performance :-
→ Test method X more sensitive & specific than Y for diagnosis of d'se

eg : PSA Vs PAP

(3) Clinical Impact :—

Diagnostic :- ~~In How much cases → diagnostic~~
↳ How the test causes change in clinician diagnostic capability

eg :-
~~Enzyme~~ Urea is v. sensitive markes for Δ s/c of hypovolemia
↓
But doesn't provide any more addition Informan
↓
By looking / clinical symptom → pt hypovolemic is diagnosed

Therapeutic :-
→ Does test provide useful informan about change in thesapy
eg :- Blood glucose level ↓ effect on Rx → Oral Hypoglycemic or insulin

Health outcome :-

↓

PSA diagnose prostatic Ca. at early stage But early diagnosis may not result in improvement in mortality.

(4) Organizational Impact :-

eg:- mammography in Breast cancer is beneficial By health outcome

→ Tropnin in MI is useful By health outcome

↓

But although is more useful for actual outcome B/w two test → Organizational impact → so that will be done

(5) Cost effectiveness :-

comparing the cost of test & cost of not doing the test

eg:- certain lab. diagnosis → causes shortes hospital stay compared & that person earning

(6) Decision :-

↳ Taken By nation → To divest fund to appropriate person

eg:- TB diagnosis → Only sputum & X-Ray is required to initiate Rx

* Multiple Univariate :-

→ for diagnosis → use more than one test

- LFT : any of 3 is abn → then dies d/se

if we consider

ALP, ALT, Bilirubin

→ if one abn → sensitivity ↑ But speafiaty ↓

→ Critesia for TB diagnosis c include ADA, S. protein, ~~Alb~~

~~Multiple, Univaral~~

* Multivariate :-

Multiple, univariate :-

(2)

Clinical Application of MS

① GC-MS :—

→ Use as a ref. method for → glc, cHO, Ca, BUN

→ Use for drug testing for drug & forensic purpose

↓

it is suitable for that who has
- small mw
- nonpolar
- volatile properties

} Most useful is EI + TOF

→ Unknown compound can be identified by comparing their full mass spectrum to mass spectral library.

→ Numerous xenobiotic compounds can be analyzed
→ Anabolic steroid
→ pesticides
→ pollutant
→ Inborn error of metabolism

} Can be detected.

→ Requirement in GC-MS is the compound shoud be sufficiently volatile to allow transfer from solid phase to mobile carrier gas & elute from the colum to detector.

→ Too polar / too large compound must be chemically derivatized for use in GC-ms.

→ Fast & effective sepon can be achieved
→ excellent limit of quantification & reproducibility

(d) LC-MS :-

→ LC coupled ~~c~~ ESI & APCI :
- large mol. wt compound such as proteins can be analyzed.

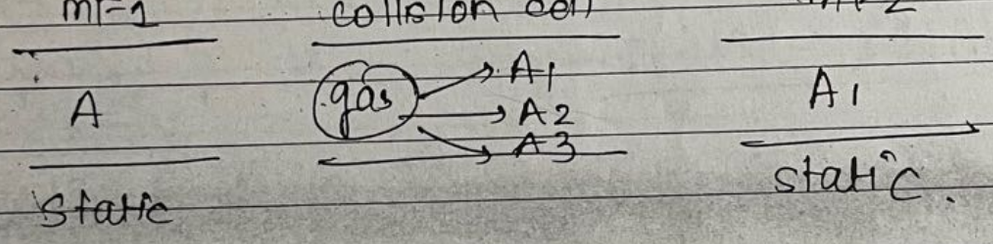
→ LC-ms/ms :-
- useful in toxicological screening & conformation
- MF1 looks for large range of unnecessary precouse ion
↓
To avoid this → TMS can be set into NRM mode
↓
So selected precoussor product - precoussor pair can be analyzed
↓
that coveas drugs rouge.

→ ~~TOF~~ kC/LC + TOF :-
↓
- also useful for toxicological screening
- High mass resolution
- Can acubately measure even low quantity
- Need for compound fragmentation is minimized.

→ LC-MS/MS:—

- Can be useful to detect →
  - immunosupressant drugs
  - Biogenic amines
  - Anti-Retroviral drug
  - Thyroid hormon
  - psychoactive drugs.

→ For quantification of specific compound most effective approach is MRM ~~mod~~ analysis in static mode.

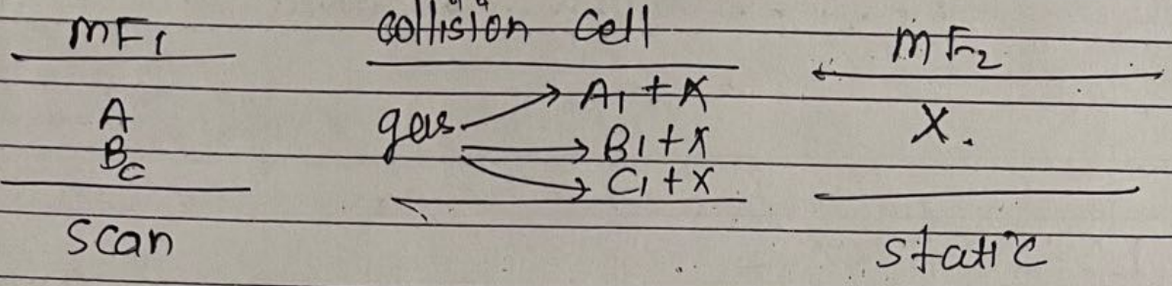


→ Screening & conforma" of genetic disorder & inborn error of metabolism:

→ ESI-MS/MS :— ~~MRM~~ Precoussor ion mode scan is used :—

→ has become recognized ref. method for carnitin & Acylcarnitine analysis to identify organic acidemia & FA oxidation defects.

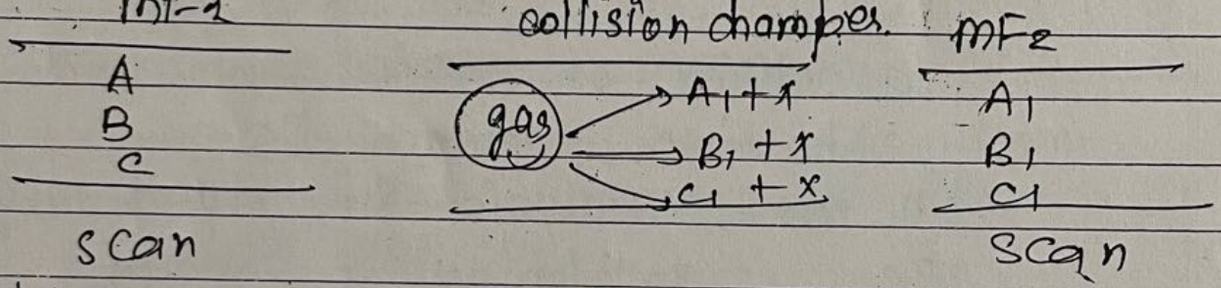
→ desivatiza" of acylcarnitin & butylesters is required.



→ X- Amino acid shares a common neutral product — Butyl Formate

↓

So they can be seperated by using "constant neutral loss scan"



③ NAIDI - MS :—

→ For detection & analysis of protein & peptide

→ Usually coupled ~~&~~ MS - TOF-

→ Identified or organism like Bact By peptide Mass Fingerprinting

④ ICP - MS :—

→ for determination of toxic element & trace mated.

ASCO :- American society of Clinical oncology

EGTM :- European group of tumor markers

NACB :- National academy of clinical biochemistry

Breast Cancer:

In all ASCO, EGTM, NACB:

(1) ER, PR : For predicting response to therapy (Tamoxiphen)

HER-2 : (+ve Herceptin)

net { ER, PR, HER

(2) CEA, CA-15-3, CA-27-29 } for monitoring Rx → ↑ suggest Rx failure

(3) UPA/PAI-1 → for (Prognosis of) node -ve cancer.

Urokinase plasminogen activator, Plasminogen Activator Inhibitor

(4) Oncotypes DX → node -ve, ER +ve } Rx + tamoxifen

Predicting Recurrence

Ovarian Ca:

↓

CA-125 : Asis, monitoring, therapy, prognosis, Recurrence.

Prostate:

↓

PSA & DRE (Digital Rectal examin")

↓

For early detec", prognosis, monitoring

→ % FPSA : when PSA – 4 to 10 µg/L &
-ve DRE

* Germ cell :—

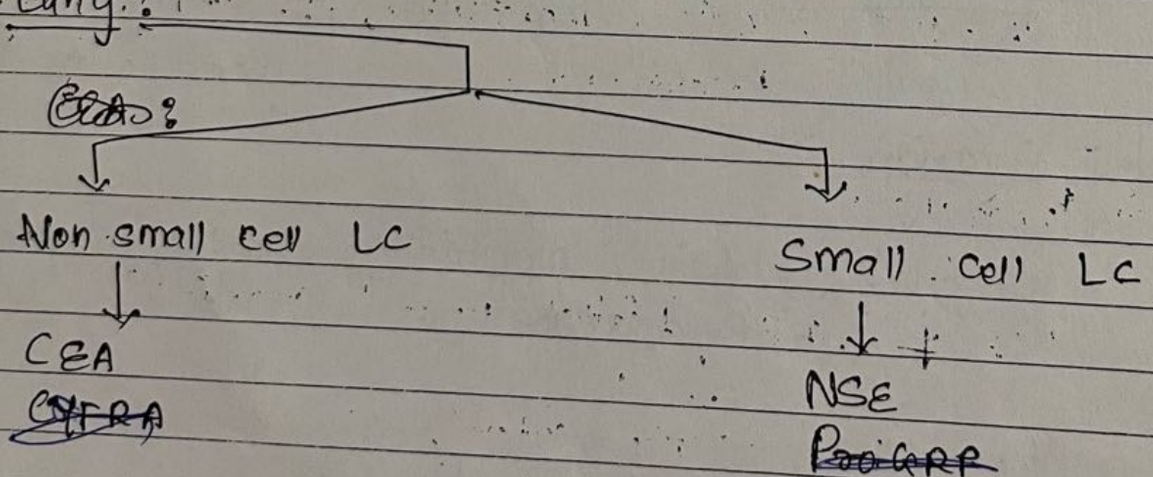
Testicular Tumor : AFP, hcg, LD
↓
For all → use.
(Asis, prognosis, monitoring ob
therapy, reaurence)

Seminomatous → Sperm cell Tumor
Nonseminomatous → embryonal cell Tumor.

* Colon :

CEA :- monitoring or advanced dise,
prognosis
surveillence
(Not for Asis)

* Lung :



* ER & PR :

→ are found in breast cancer cells that depends on estrogen & related hormon to grow.

→ All pt ⊂ invasive Breast Ca. / Breast Ca recurrence should have their tumors tested for three receptors.

→ Breast Cancer
↓
ER/PR
↓ ↓
+ve -ve
↓ ↓
ER/PR +ve ER/PR -ve
↓ Rx ⊂
Hormone thesapy → Blocks the tumor using estrogen
By Rx

→ Testing of ER/PR → Helps to guide thesapy
↓
2 Types of drugs
↓ ↓
Tamoxifen Aromatase inhibitor
$

→ Immunohistochemistry is useful for detecting → ER / PR

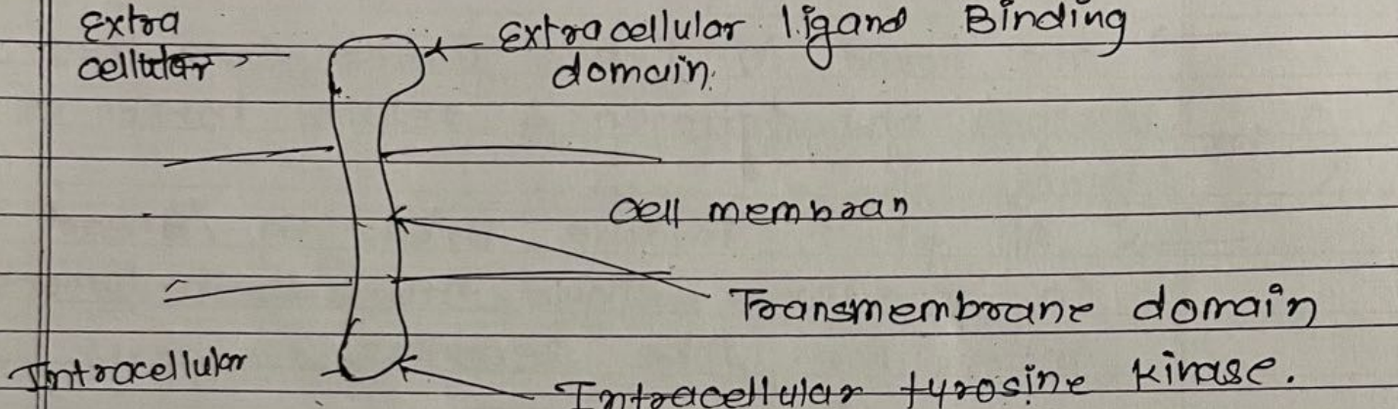
HER-2 :

(Human Epidermal growth factor 2 → ligand for HER-2 receptor

↓

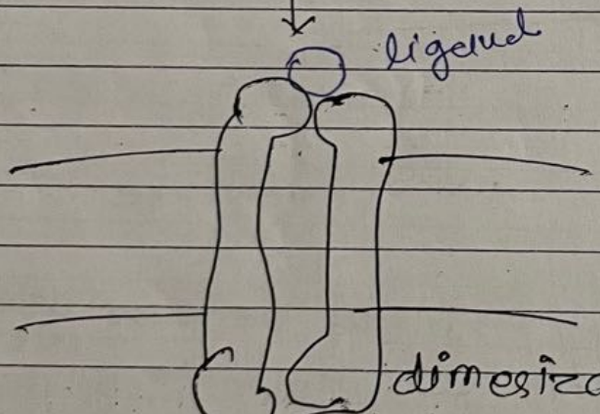
It is a part of EGF family

↓



(HER-2 receptor)

↓



dimeriza^{n} of HER-2 (without Herceptin)

↓

Signal to

↓

- Cell proliferation.
- Cell differentiation

↓

Cancer cell have gene mutation that make excess of HER-2 protein

↓

Uncotrolled growth

→ HER-2 +ve Breast cancer

↓

- more aggresive
- worst prognosis

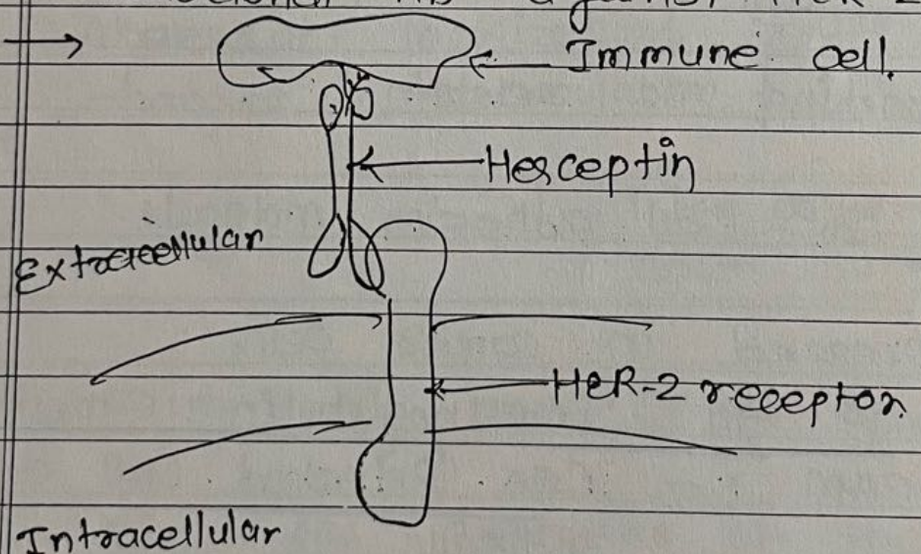
→ Rx :-

Transtuzumab : Hescaptin :-

↓

→ monoclonal Ab against HER-2 receptor.

→



→ Q. Hescaptin is only approved HER-2 Therapy designed to bind to HER-2 +ve tumor cells ..

↓

& flag them for destruo^n By immune system.

↓

Immune cells will target cell that are bound by Herceptin.

↓

Herceptin blocks downstream HER-2 signaling

↓

to inhibit proliferation of cell.

* Carcino Embryonic Ag :- [CEA] Colon non small cell lung Breast ca

↓

→ glycoprotein mol.

→ CEA is produced during development of fetus

↓

Stops before Birth

↓

Not tnt in healthy individual.

→ it is GPI anchored protein

↓

working as selectin

↓

Ig as cell adhesion moleaule.

→ Expressed in cancer cell.

→ Belongs to Immunoglobulin superfamily

→ CEACAM :- CEA Related Cell adhesion moleaule

* CA 15-3 :-

→ Cancer Antigen 15-3

→ MUC-1 :- Mucin-1 cell surface associated
↓
→ glycoprotein & O-linked glycosylation
→ this protein is anchored to the apical surface of many epithelia by transmem. domain like lung, colon, stomach, ~~Bone~~

→ Function:
↓
- Serves protective function by binding to pathogen
- Cell signaling

→ Overexpression
Abnormal localization
changes in glycosylation
} ass. & carcinoma,
↓
like Ovary, Breast, colon, lung, pancreas.

→ CA 15-3 & ass. CA 27-29
↓
- are diff. epitopes on the same protein Ag ~~with~~ product of Breast Cancer associated MUC-1 gene

* CA-125 :

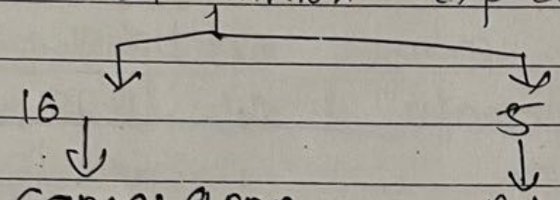
↓

- Encoded by MUC-16 gene
- glycoprotein mucin

* Oncotype dx :—

→ Test of mRNA expression

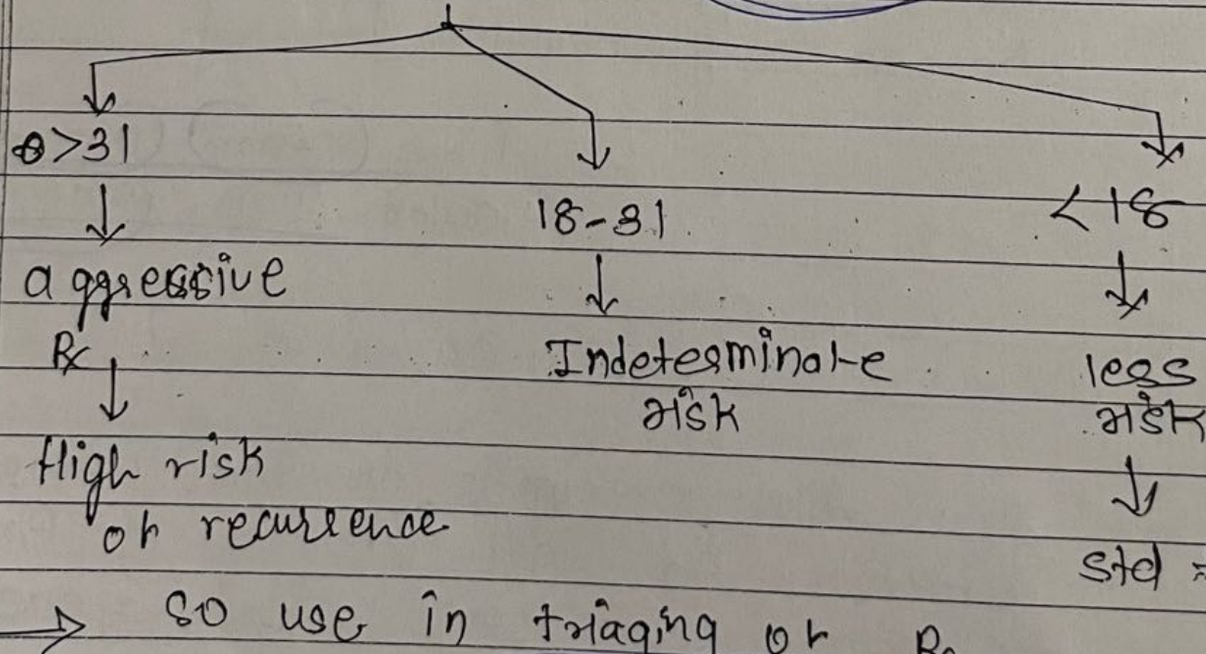
⇒



→ Use in women c early stage, Node -ve, ER +ve } Breast Ca.

→ Assay provide assessment of response to chemotherapy & of recurrence in 10 yrs.

→ Recurrence score (0-100)



→ So use in triaging of Rx

* Mammaprint :-

→ DNA microarray of 70 most significant gene for breast cancer.

* PSA :- / Kallikrein-3 :-

→ glycoprotein
→ Protease enzyme → lyse seminogelin & fibronectin in sperm coagulum
→ liquifies semen in semen coagulum & allow sperm to swim freely
→ dissolve the cervical mucus
↓
facilitates sperm entry.

* Free PSA :-

→ most PSA in the bound to serum protein.
→ small amount not bound to protein
↓
K/a "free PSA"
→ In men ∈ prostate cancer
↓
ratio of free/Total PSA is ↓ed
↓
→ In men ∝ PSA level 4 to 10 ng/ml
↓
Measuring the ratio of free/total PSA
↓
: appears to be useful for eliminating unnecessary Biopsies.

* AFP :- function unknown

→ protein encoded by AFP-gene lnt on q arm oh chromosome 14

→ AFP is a major plasma protein produced by the yolk sac & lives during fetal development.

→ AFP binds to copper, Nit, FA & Bilirubin

→ found in monomesic, dimesic, trimesic form.

* Inactive PSA :-

↓

~~marker~~ cell

Cancer^ produce & altered/ Proenzyme form of PSA

↓

more indicative of indicator oh canc.

→ DPM $\propto \frac{1}{\text{sigma}}$