ΔΗΜΟΚΡΙΤΕΙΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΡΑΚΗΣ ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ

ΤΜΗΜΑ ΜΟΡΙΑΚΗΣ ΒΙΟΛΟΓΙΑΣ & ΓΕΝΕΤΙΚΗΣ

ΜΕΤΑΦΡΑΣΤΙΚΗ ΕΡΕΥΝΑ ΣΤΗ ΒΙΟΪΑΤΡΙΚΗ – Μοριακή Διαγνωστική, Βιοδείκτες και Στοχευμένες Θεραπείες

Γονιδιωματική και βιοδείκτες στον Καρκίνο

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Εθνικό και Καποδιστριακό
Πανεπιστήμιο Αθηνών

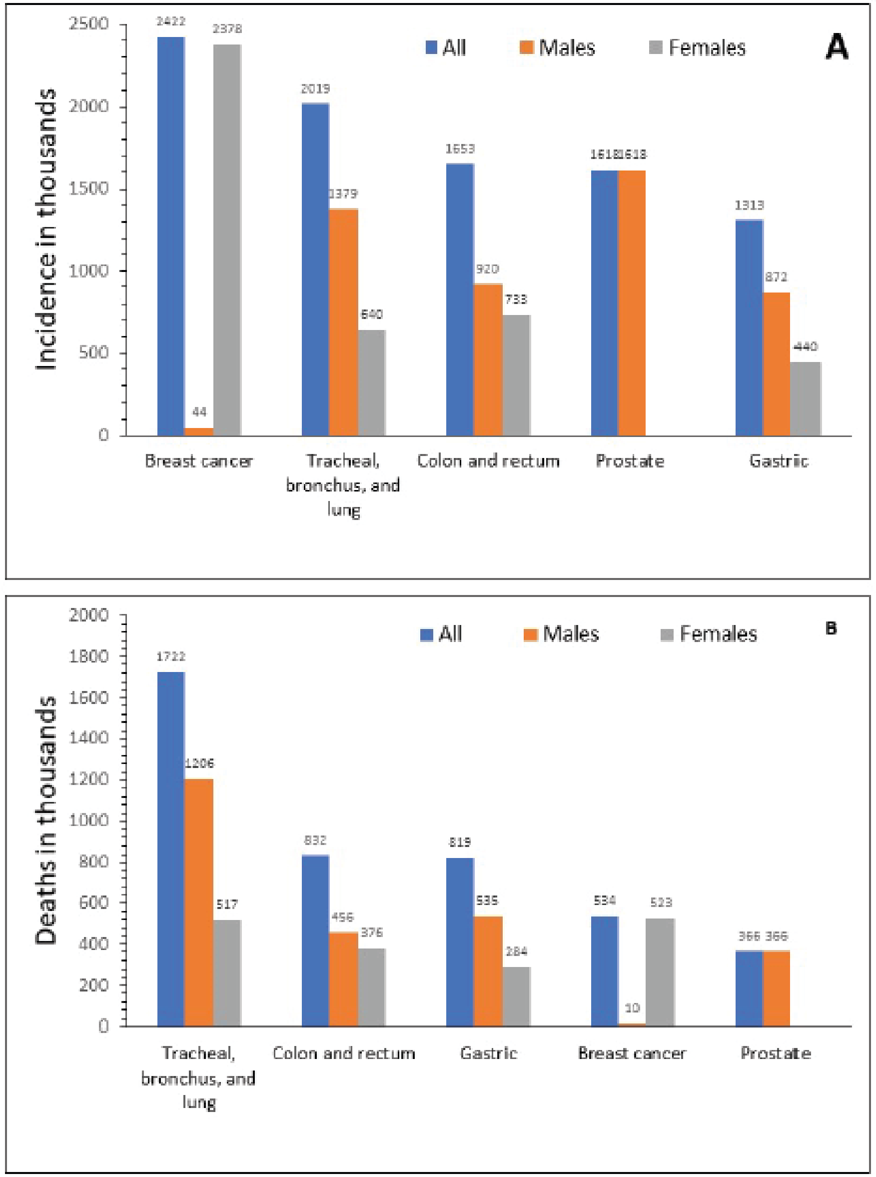
Epidemiological data

Over 1 million estimated new cases annually, gastric cancer is the **fifth most diagnosed malignancy** worldwide.

Mortality is high, making it the **third most common cause of cancer related deaths**, with 784,000 deaths globally in 2018.

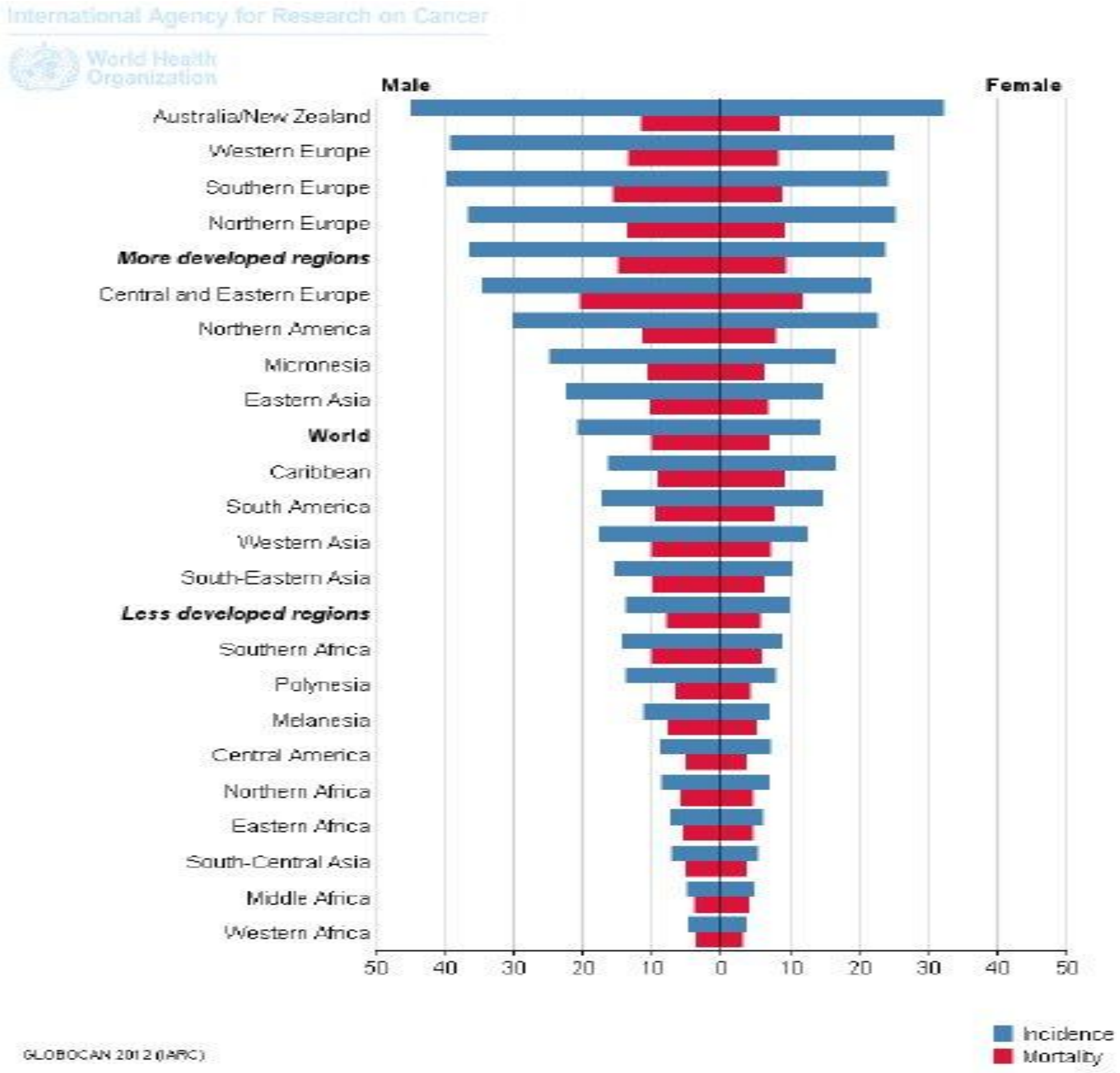
Montserrat Casamayor, et al. Ecancer medical science. 2018
Bray F, et al. CA Cancer J Clin 2018; 68: 394–424.

Global incidence (a) and mortality (b) for different types of cancer in 2015.



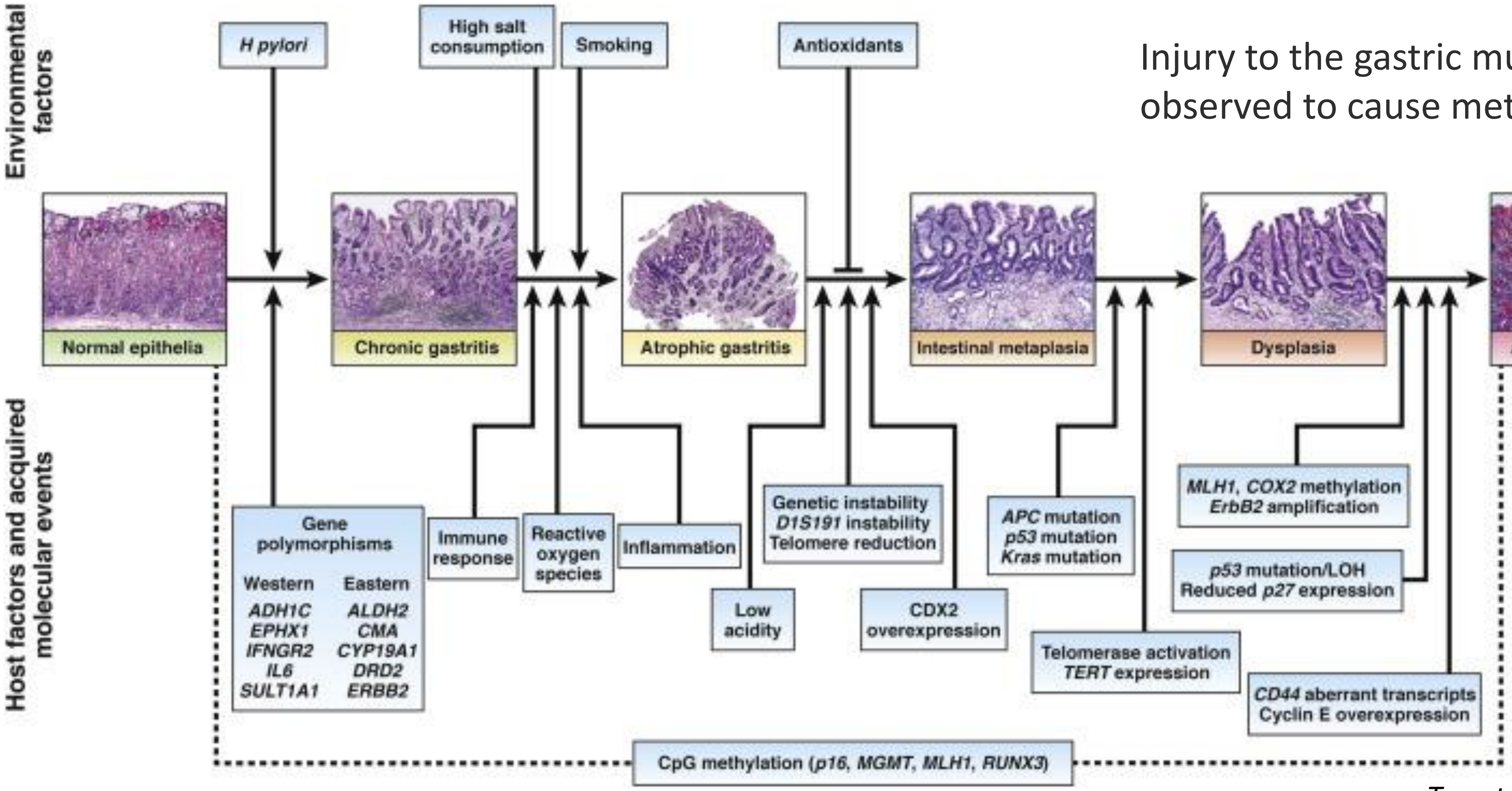
Epidemiological data

- The incidence of gastric cancer is **two times higher in males than in females**.
- **Hotspots of incidence and mortality** for gastric cancer exist in Europe, and Australia/New Zealand.
- The average age of people when they are diagnosed is 68.
- Despite declining incidence rates in most countries, clinicians can expect to see **more gastric cancer cases in the future** due to aging populations.



GLOBOCAN 2012 (IARC)

Complex Disease Pathogenesis

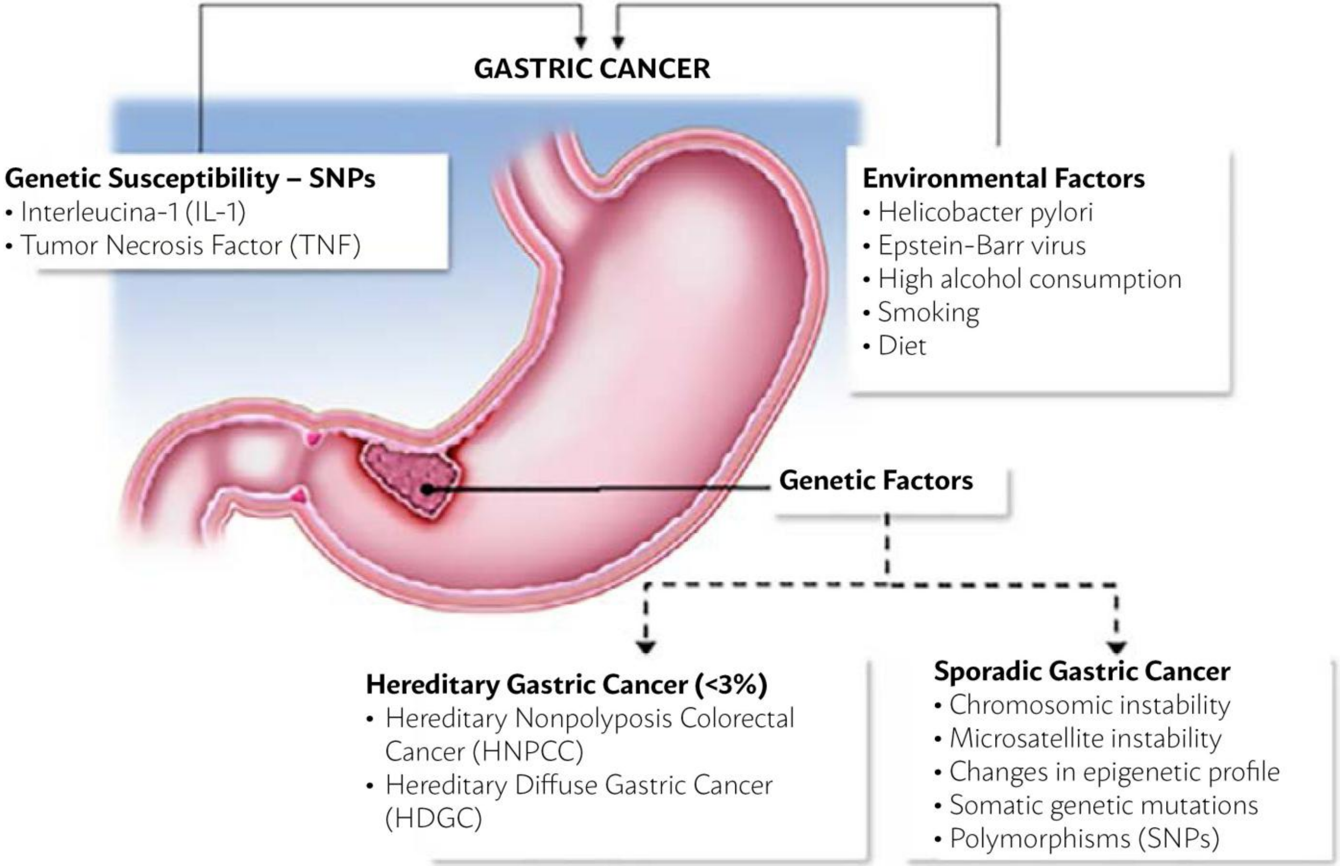


Injury to the gastric mucosa has also been observed to cause metaplastic changes

Tan et al. Gastroenterology. 2015

Risk factors of Gastric Cancer

inflammation, infection, and tumors



GASTRIC CANCER

Genetic Susceptibility – SNPs
- Interleucina-1 (IL-1)
- Tumor Necrosis Factor (TNF)

Environmental Factors
- Helicobacter pylori
- Epstein-Barr virus
- High alcohol consumption
- Smoking
- Diet

Genetic Factors

Hereditary Gastric Cancer (<3%)
- Hereditary Nonpolyposis Colorectal Cancer (HNPCC)
- Hereditary Diffuse Gastric Cancer (HDGC)

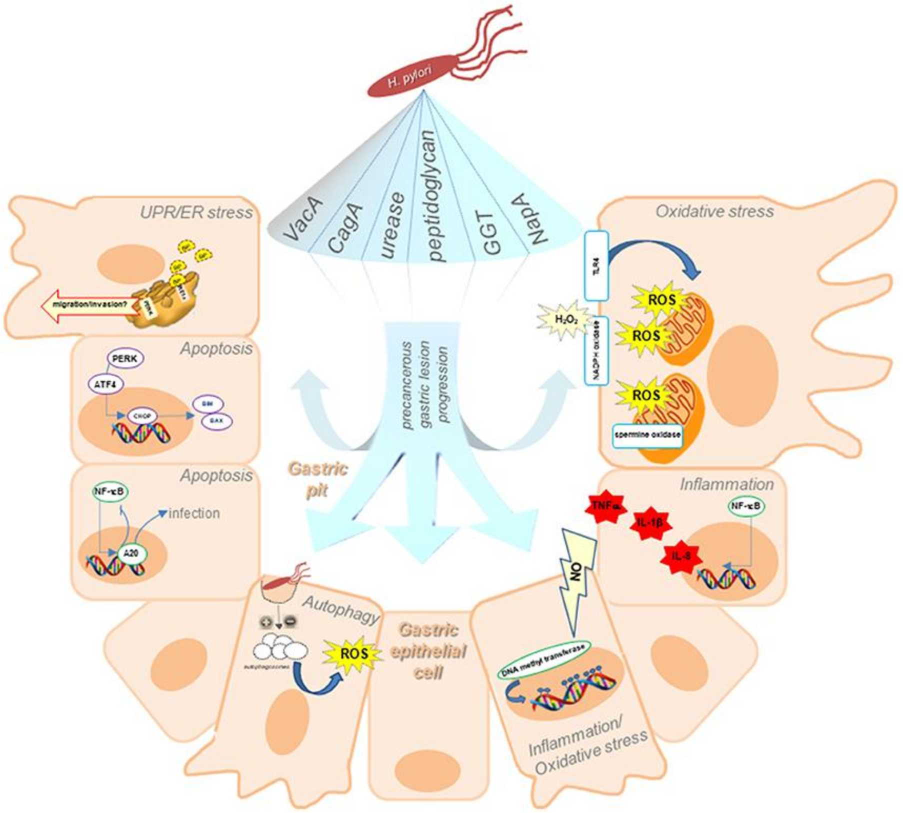
Sporadic Gastric Cancer
- Chromosomic instability
- Microsatellite instability
- Changes in epigenetic profile
- Somatic genetic mutations
- Polymorphisms (SNPs)

Ramos et al. Rev. Assoc. Med. Bras. 2018

Environmental factors in GC

Helicobacter pylori

- Carcinogenic effect through the **CagA protein** (immunogenic antigen).
- 100% of infected Asian and 70% of US patients express the CagA protein.
- CagA activates a signaling cascade, either SHP2, Abl, or Src kinases, within the gastric cancer cell.
- Polymorphisms of the CagA protein are associated with the **development and incidence rate of gastric cancer**.
- Upregulation of various pro inflammatory cytokines such as IL-8 and COX leading to **chronic inflammation and cancer development**..
- Secretes the **VacA toxin**, a compound which can **suppress T-cell responses**, allowing lesions to form with little push back from the immune system.
- Individuals with eradication of *H. pylori* infection had a **lower incidence** of GC.



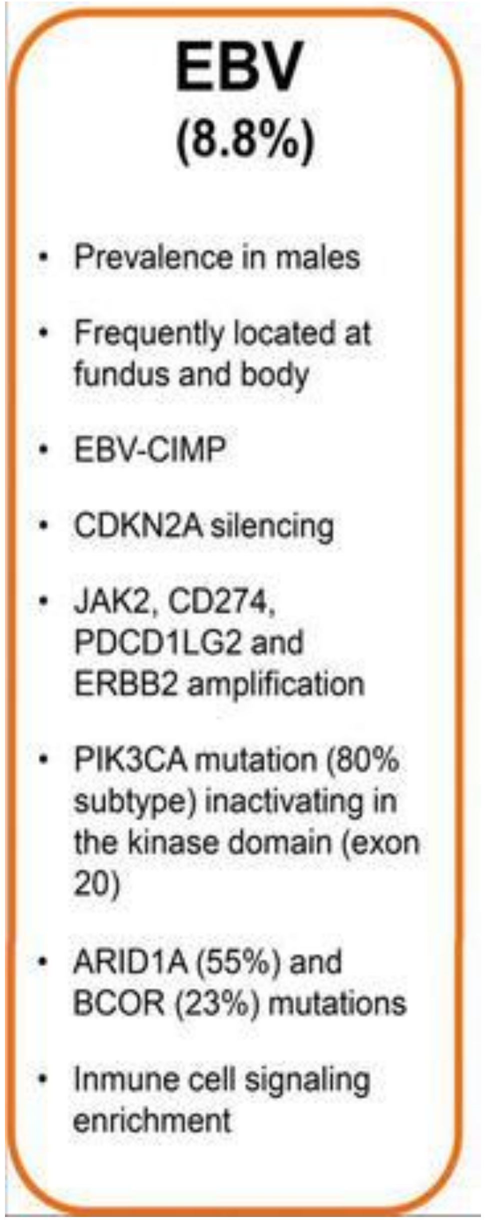
Sexton et al. Cancer Metastasis Rev. 2020

Diaz et al. Front. Microbiol. 2018

Environmental factors in GC

Epstein Barr virus (EBV) has also been shown to influence GC progression in a subset of cases (10%).

- **Only CD21^{high} cells are vulnerable to EBV infection** - B cells and follicular dendritic cells, but also T cells.
- Prompts **methylation** of the host genome (CpG island methylator phenotype - CIMP), **imbalance** of the cellular signaling pathways, generation of a **tumor microenvironment** of infected gastric epithelial cells.
- Is now considered a unique molecular subtype of gastric cancer and is associated with **good prognosis** in patients.



EBV
(8.8%)

- Prevalence in males
- Frequently located at fundus and body
- EBV-CIMP
- CDKN2A silencing
- JAK2, CD274, PDCD1LG2 and ERBB2 amplification
- PIK3CA mutation (80% subtype) inactivating in the kinase domain (exon 20)
- ARID1A (55%) and BCOR (23%) mutations
- Inmune cell signaling enrichment

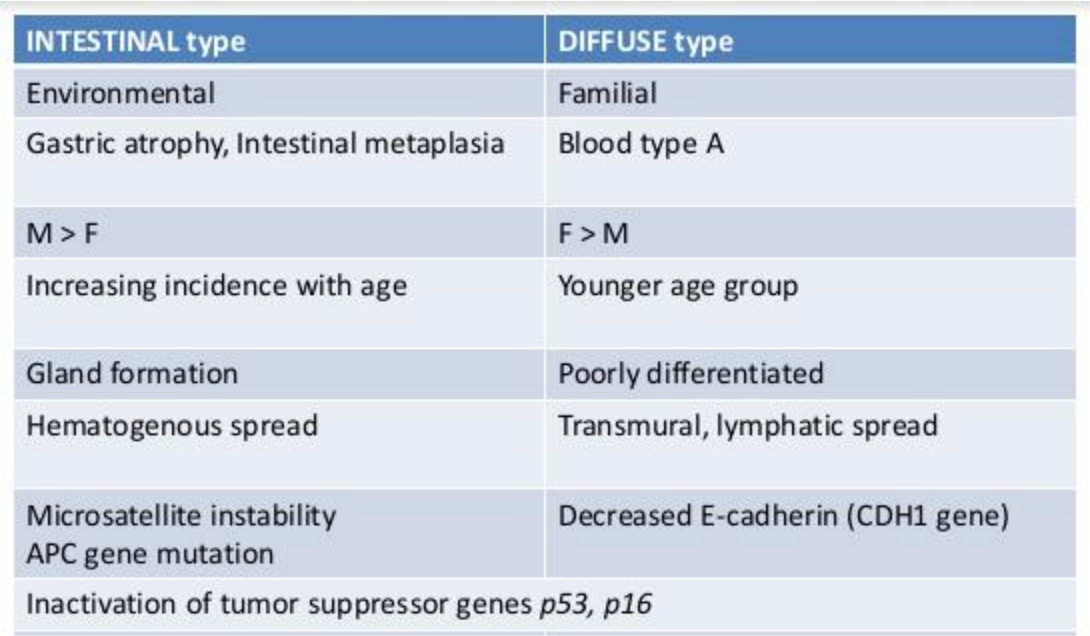
Sexton et al. Cancer Metastasis Rev. 2020

Histological classification of GC

Traditionally, GC classification has been based on histopathological and morphological features.

Lauren classification (1965):

I. intestinal-type gastric cancer (IGC) – 53%,
II. diffuse-type gastric cancer (DGC) – 33%,
III. mixed/indeterminate subtypes – 14%.



INTESTINAL type	DIFFUSE type
Environmental	Familial
Gastric atrophy, Intestinal metaplasia	Blood type A
M > F	F > M
Increasing incidence with age	Younger age group
Gland formation	Poorly differentiated
Hematogenous spread	Transmural, lymphatic spread
Microsatellite instability APC gene mutation	Decreased E-cadherin (CDH1 gene)
Inactivation of tumor suppressor genes *p53, p16*	

tumor suppressor gene

cell adhesion

These subtypes besides differing in terms of **risk factors** they display a distinct **clinical prognosis**, where patients with **DGC typically experience poor prognosis, poor response to treatment and shorter overall survival**.

Lauren P. Acta Pathol Microbiol Scand. 1965

Histological classification of GC

- WHO classification:

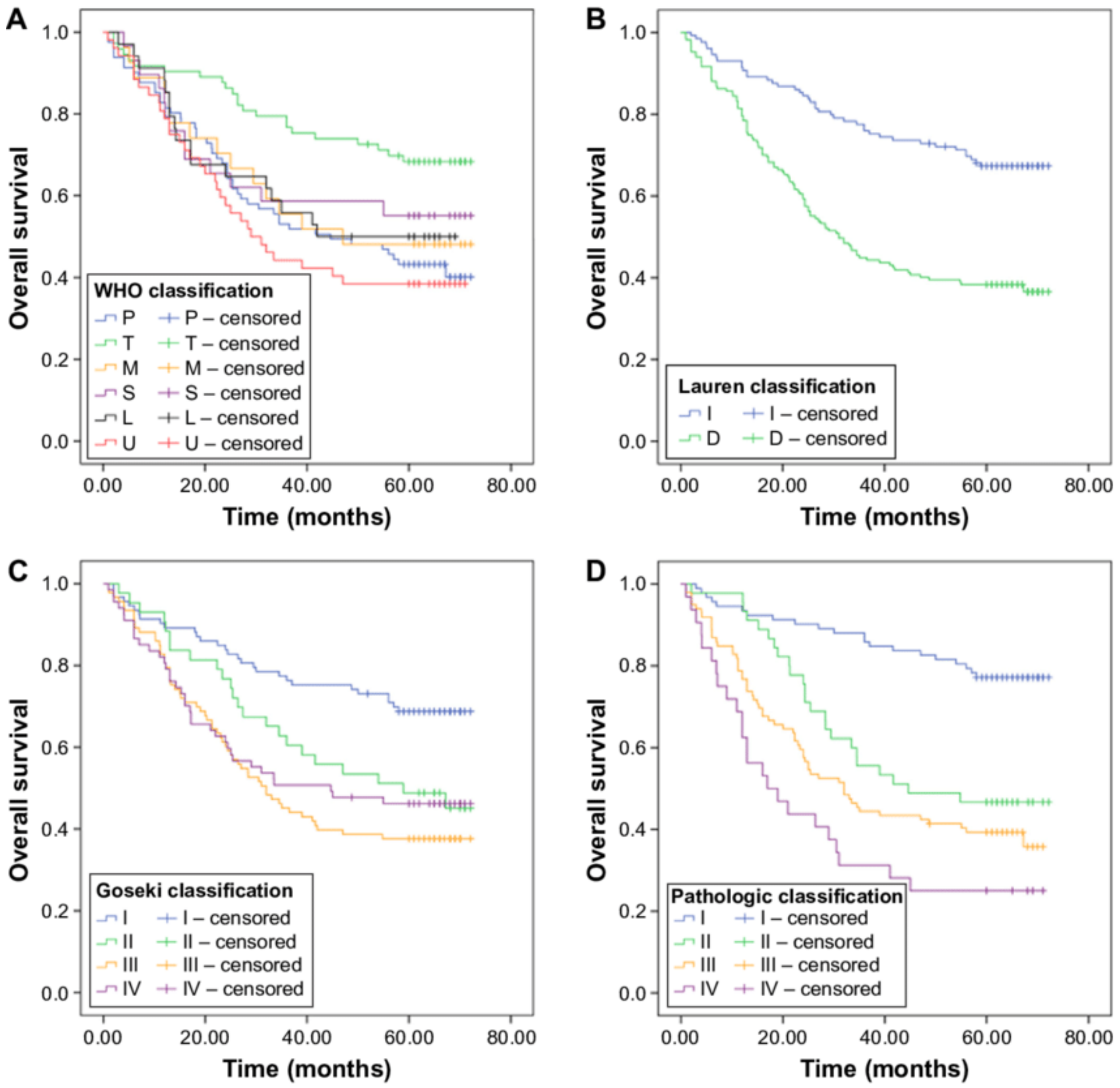
I. tubular,

II. papillary,

III. mucinous,

IV. poorly cohesive/differentiated and signet ring cell subtypes

Hamilton SR, et al. Lyon: IARC Press; 2000



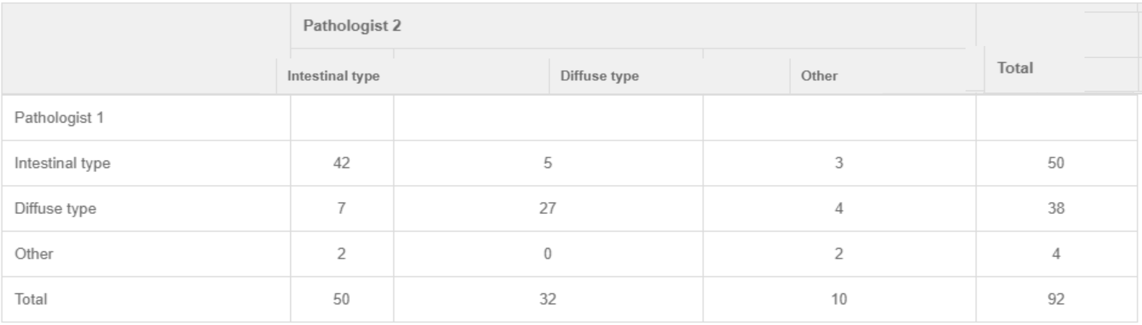
Che et al. OncoTargets and Therapy. 2017

Issues of Histological classification of GC

- A greater understanding of the **molecular changes** associated with gastric cancer is needed.
- Current histopathologic systems remain insufficient to **guide precise treatments** for individual patients.
- Traditional classifications are unable to **identify actionable molecular targets**.

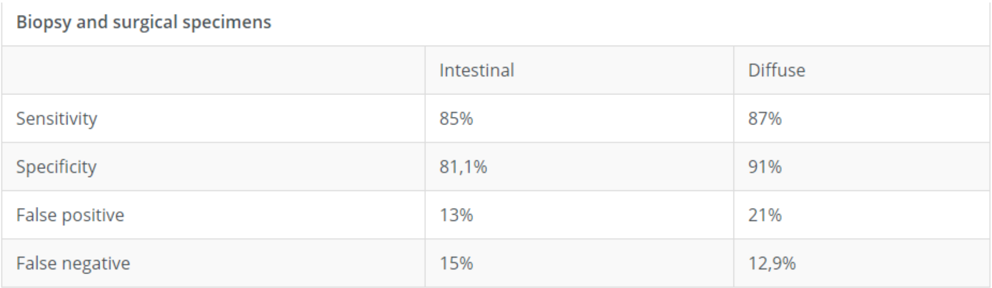
Can be used to guide patient selection for targeted therapy, identifying alterations with a higher impact on outcome based on available strength of evidence.

Overall concordance in histological classification between pathologists[a]



	Pathologist 2			Total
	Intestinal type	Diffuse type	Other	
Pathologist 1				
Intestinal type	42	5	3	50
Diffuse type	7	27	4	38
Other	2	0	2	4
Total	50	32	10	92

- [a] Observed concordance = (42 + 27 + 2)/92 = 77%. κ coefficient = 0.59 (95% CI, 0.44–0.73).



Biopsy and surgical specimens		
	Intestinal	Diffuse
Sensitivity	85%	87%
Specificity	81,1%	91%
False positive	13%	21%
False negative	15%	12,9%

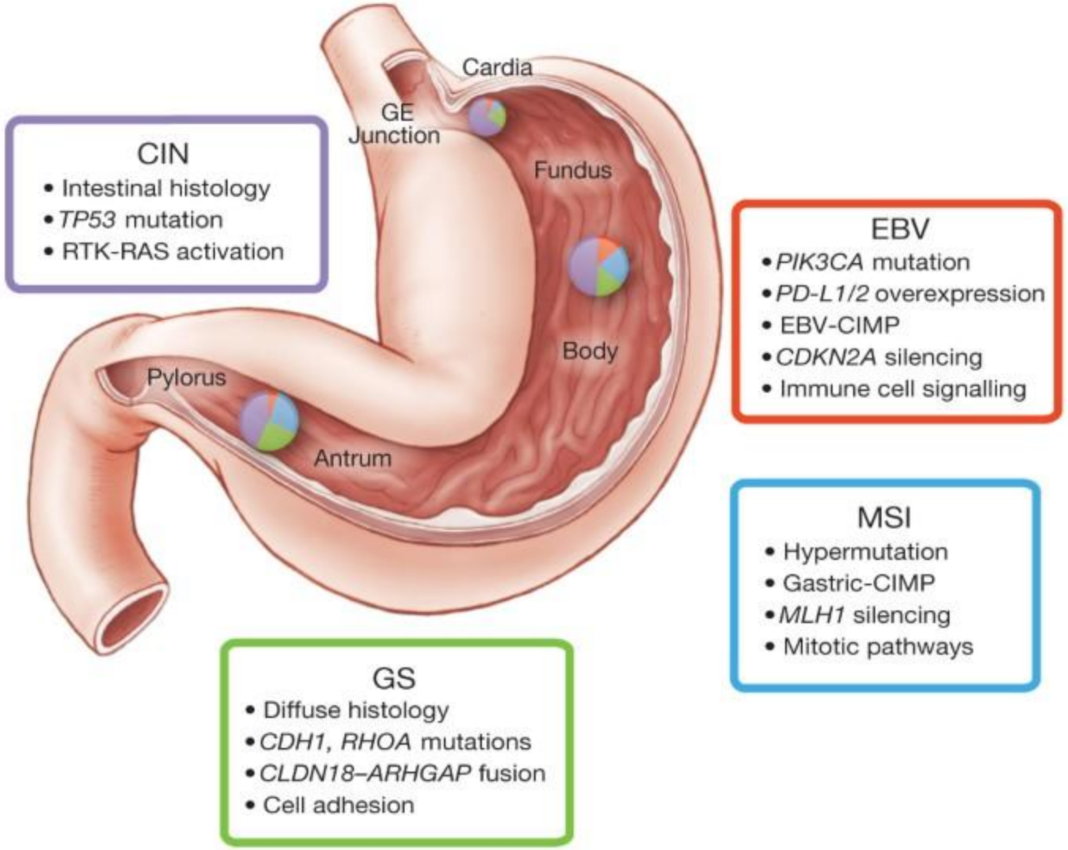
Molecular classification by TCGA

The Cancer Genome Atlas (TCGA) network

New perspectives both for patient stratification and trials of targeted therapies.

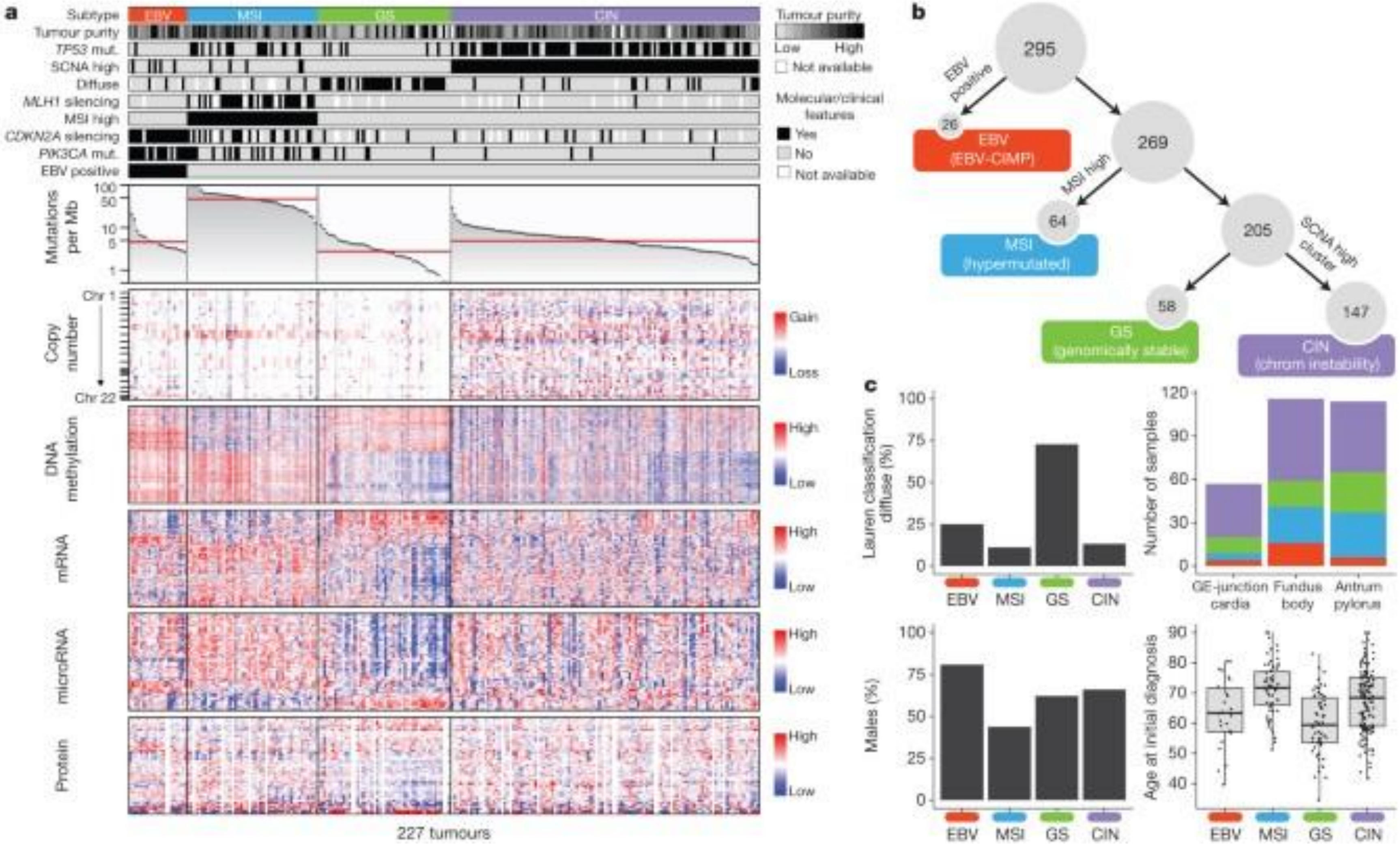
TCGA classification (>290 primary tumors of the stomach):

1) EBV-positive tumors - 9%;
2) tumors with micro-satellite instability (MSI) - 22%;
3) genomically stable (GS) tumors - 20%;
4) tumors with Chromosomal INstability (CIN 50%), which show marked aneuploidy and focal amplification of receptor tyrosine kinases.



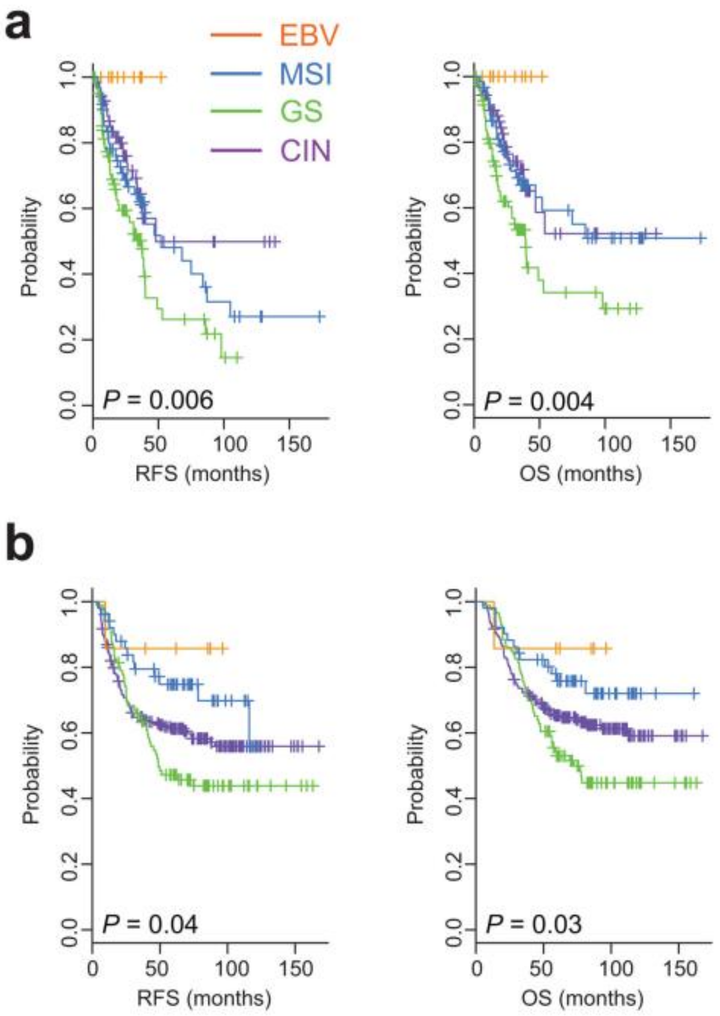
The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014

Molecular classification by TCGA



The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014

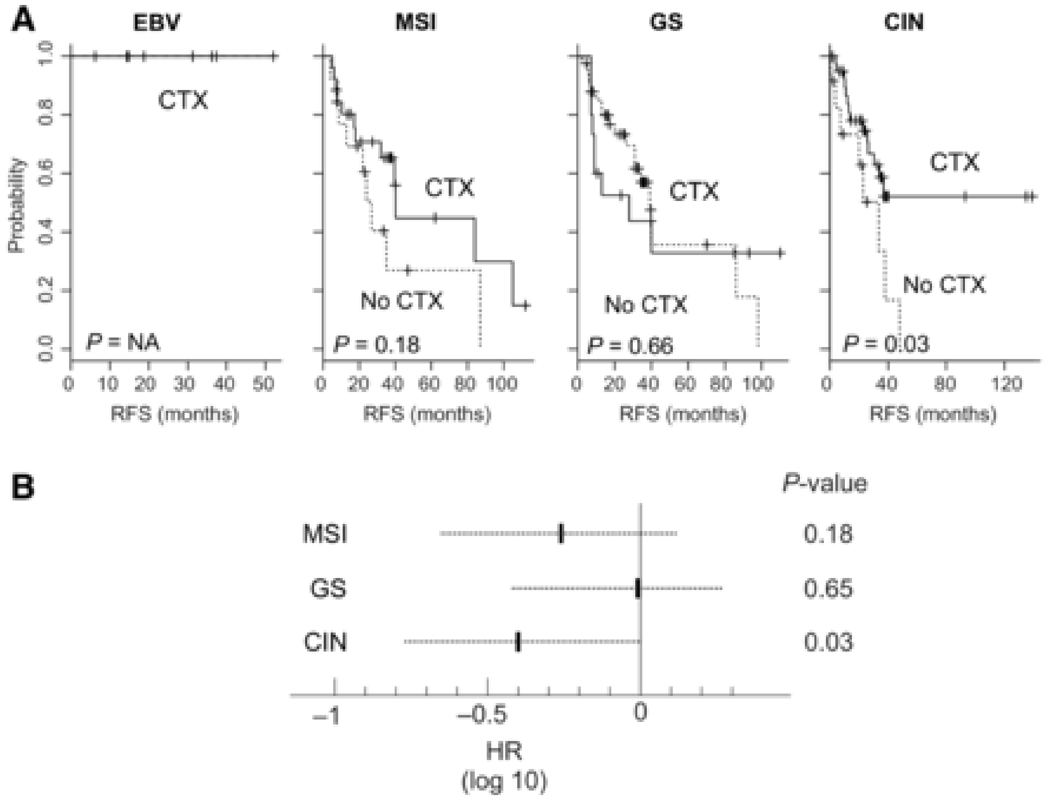
Clinical implications



Unique molecular features that could guide therapeutic decisions.

Prognostic significance, with EBV being associated with the best prognosis, and GS with the worst.

Benefit of chemotherapy among patients with each subtype of GC.



Prognosis associated with each of the 4 subtypes of GC in 2 independent patient cohorts
Patients in the MDACC cohort (A) and SMC cohort (B) were stratified by subtype **recurrence-free survival (RFS)** and **overall survival (OS)** were plotted for each subtype.

Sohn et al. Clin Cancer Res. 2017

Molecular classification by ACRG

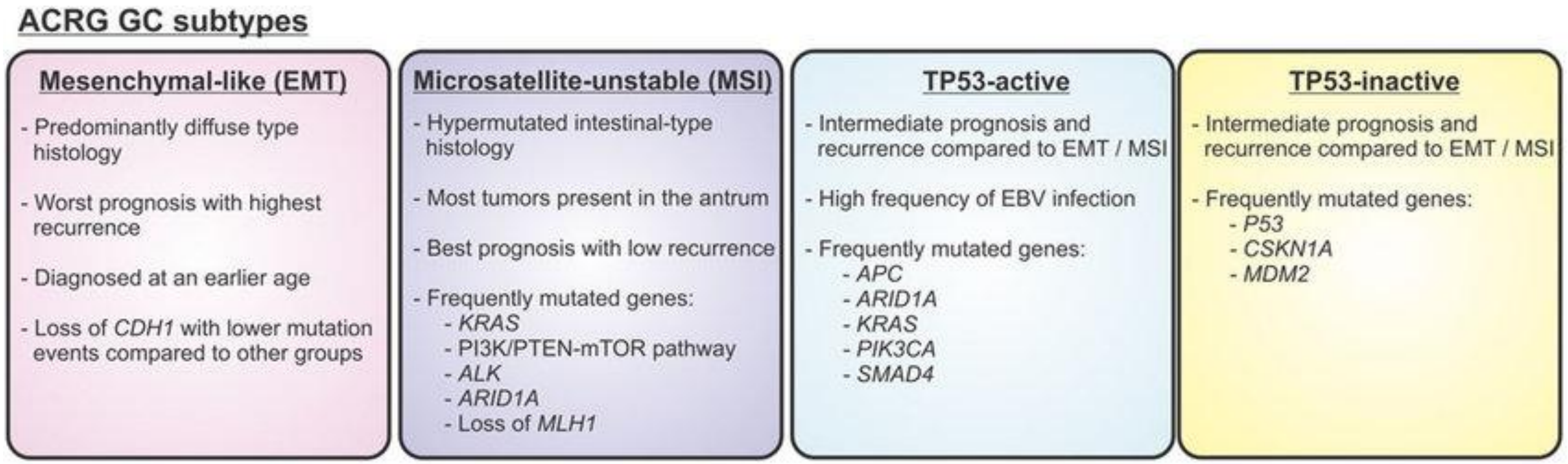
The Asian Cancer Research Group (ACRG) analyzed 300 gastric tumor samples by 2 molecular platforms and identified 4 subsets of patients:

(1) MSI (23%). Best prognosis.

(2) MSS/EMT (micro-satellite stability/EMT, 15%). The majority of subjects (>80%) in this subtype are diagnosed at stage III/IV, highest chance of recurrence (63%).

(3) MSS/p53+ (p53 active, 26%). Best overall prognosis after MSI subtype.

(4) MSS/p53- (p53 inactive, 36%). Highest prevalence of p53 mutations.



ACRG GC subtypes

Mesenchymal-like (EMT)
- Predominantly diffuse type histology
- Worst prognosis with highest recurrence
- Diagnosed at an earlier age
- Loss of *CDH1* with lower mutation events compared to other groups

Microsatellite-unstable (MSI)
- Hypermutated intestinal-type histology
- Most tumors present in the antrum
- Best prognosis with low recurrence
- Frequently mutated genes:
  - *KRAS*
  - PI3K/PTEN-mTOR pathway
  - *ALK*
  - *ARID1A*
  - Loss of *MLH1*

TP53-active
- Intermediate prognosis and recurrence compared to EMT / MSI
- High frequency of EBV infection
- Frequently mutated genes:
  - *APC*
  - *ARID1A*
  - *KRAS*
  - *PIK3CA*
  - *SMAD4*

TP53-inactive
- Intermediate prognosis and recurrence compared to EMT / MSI
- Frequently mutated genes:
  - *P53*
  - *CSKN1A*
  - *MDM2*

Cristescu R, et al. Nat Med. 2015

The effect of the cohort

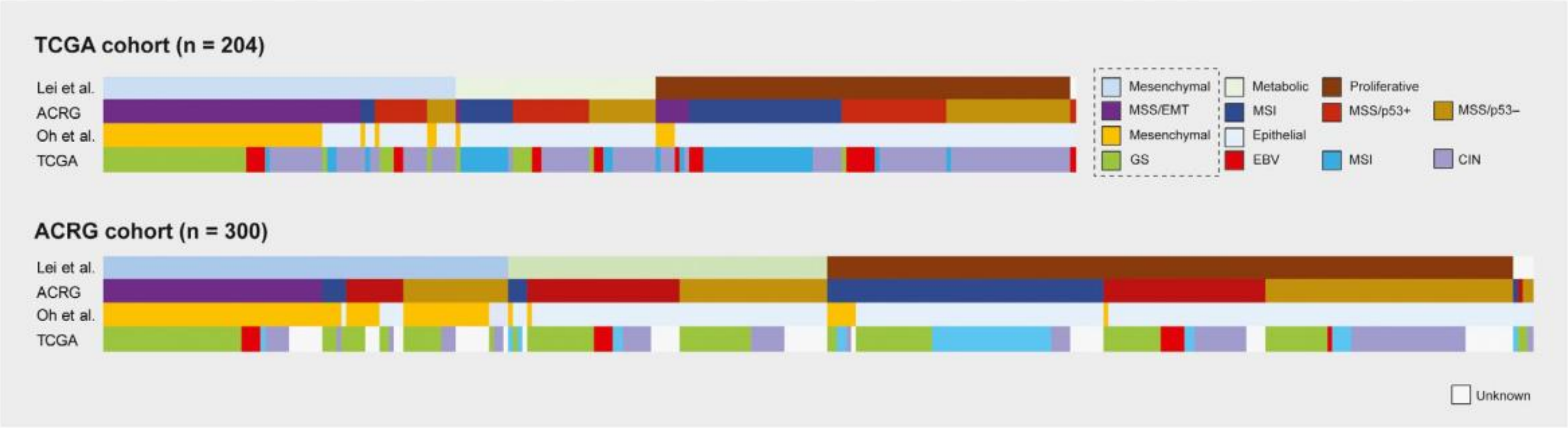
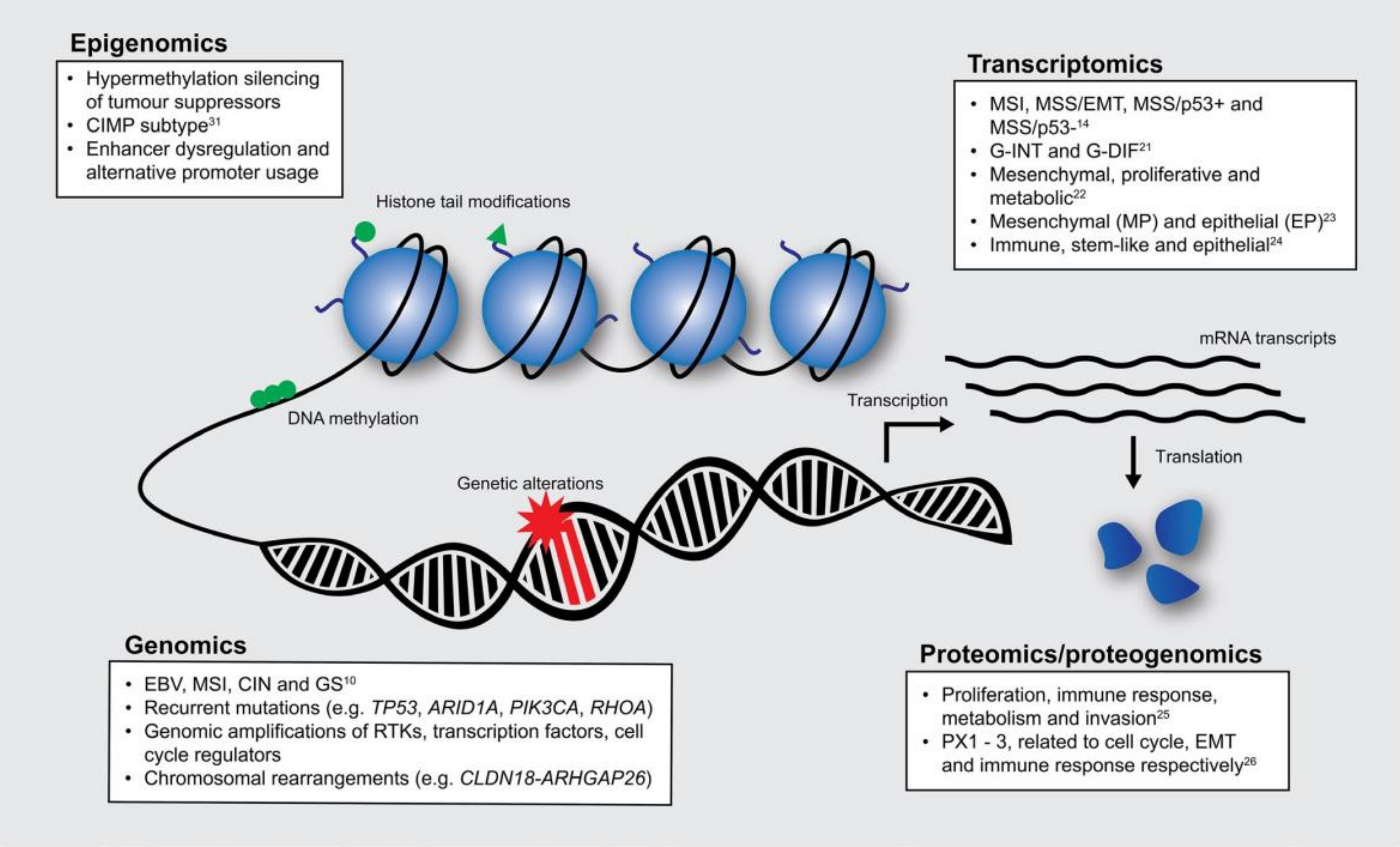


FIGURE 2 Distribution of the various transcriptomic-based (Lei et al,[22] Asian Cancer Research Group [ACRG], Oh et al[23]) and The Cancer Genome Atlas (TCGA)-based subtypes in two independent cohorts. A strong overlap is observed among the Lei et al mesenchymal subtype, ACRG microsatellite stable with epithelial-mesenchymal transition phenotype (MSS/EMT) subtype and Oh et al mesenchymal phenotype subtype. TCGA genomically stable (GS) subtype is comparatively more homogenous in TCGA cohort and overlaps largely with the transcriptomic-based mesenchymal subtypes, unlike in the ACRG cohort. CIN, chromosomal instability; EBV, Epstein-Barr virus; MSI, microsatellite instability

Ho et al. Cancer Science. 2019

Using the intrinsic cell properties of GC - omics



Epigenomics

- Hypermethylation silencing of tumour suppressors
- CIMP subtype[31]
- Enhancer dysregulation and alternative promoter usage

Transcriptomics

- MSI, MSS/EMT, MSS/p53+ and MSS/p53-[14]
- G-INT and G-DIF[21]
- Mesenchymal, proliferative and metabolic[22]
- Mesenchymal (MP) and epithelial (EP)[23]
- Immune, stem-like and epithelial[24]

Genomics

- EBV, MSI, CIN and GS[10]
- Recurrent mutations (e.g. *TP53*, *ARID1A*, *PIK3CA*, *RHOA*)
- Genomic amplifications of RTKs, transcription factors, cell cycle regulators
- Chromosomal rearrangements (e.g. *CLDN18-ARHGAP26*)

Proteomics/proteogenomics

- Proliferation, immune response, metabolism and invasion[25]
- PX1 - 3, related to cell cycle, EMT and immune response respectively[26]

Ho et al. Cancer Science. 2019

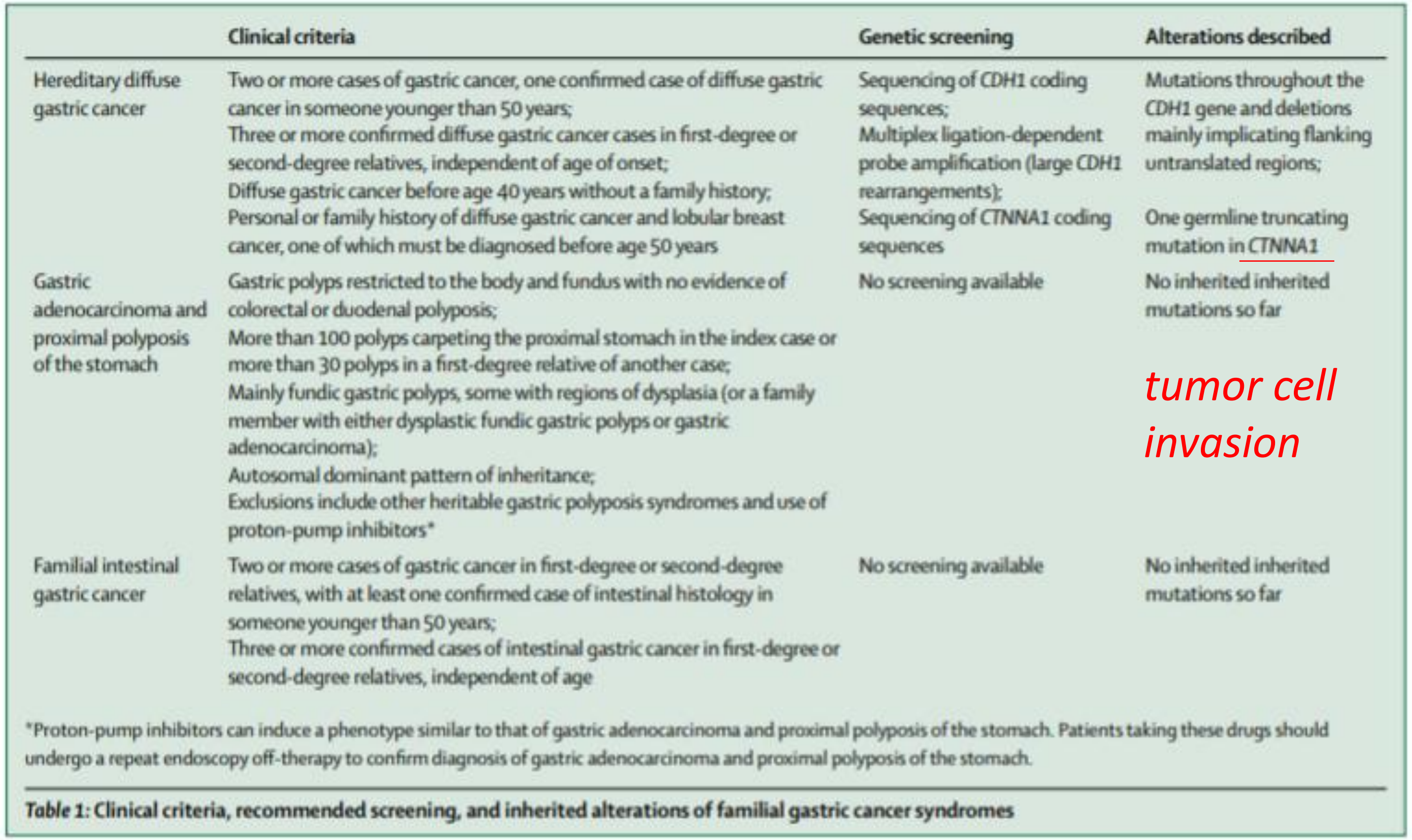
Genomics

Genomics of familial GC

1–3% of patients with gastric cancer have germline mutations.

Hereditary forms of gastric cancer can be subdivided into three groups:

I. hereditary diffuse type gastric cancer (HDGC; autosomal dominant; <1% all gastric cancer);
II. familial intestinal gastric cancer (autosomal dominant transmission of fundic gland polyposis);
III. gastric adenocarcinoma with proximal polyposis of the stomach (autosomal dominant).



	Clinical criteria	Genetic screening	Alterations described
Hereditary diffuse gastric cancer	Two or more cases of gastric cancer, one confirmed case of diffuse gastric cancer in someone younger than 50 years; Three or more confirmed diffuse gastric cancer cases in first-degree or second-degree relatives, independent of age of onset; Diffuse gastric cancer before age 40 years without a family history; Personal or family history of diffuse gastric cancer and lobular breast cancer, one of which must be diagnosed before age 50 years	Sequencing of *CDH1* coding sequences; Multiplex ligation-dependent probe amplification (large *CDH1* rearrangements); Sequencing of *CTNNA1* coding sequences	Mutations throughout the *CDH1* gene and deletions mainly implicating flanking untranslated regions; One germline truncating mutation in *CTNNA1*
Gastric adenocarcinoma and proximal polyposis of the stomach	Gastric polyps restricted to the body and fundus with no evidence of colorectal or duodenal polyposis; More than 100 polyps carpeting the proximal stomach in the index case or more than 30 polyps in a first-degree relative of another case; Mainly fundic gastric polyps, some with regions of dysplasia (or a family member with either dysplastic fundic gastric polyps or gastric adenocarcinoma); Autosomal dominant pattern of inheritance; Exclusions include other heritable gastric polyposis syndromes and use of proton-pump inhibitors*	No screening available	No inherited inherited mutations so far
Familial intestinal gastric cancer	Two or more cases of gastric cancer in first-degree or second-degree relatives, with at least one confirmed case of intestinal histology in someone younger than 50 years; Three or more confirmed cases of intestinal gastric cancer in first-degree or second-degree relatives, independent of age	No screening available	No inherited inherited mutations so far

*Proton-pump inhibitors can induce a phenotype similar to that of gastric adenocarcinoma and proximal polyposis of the stomach. Patients taking these drugs should undergo a repeat endoscopy off-therapy to confirm diagnosis of gastric adenocarcinoma and proximal polyposis of the stomach.

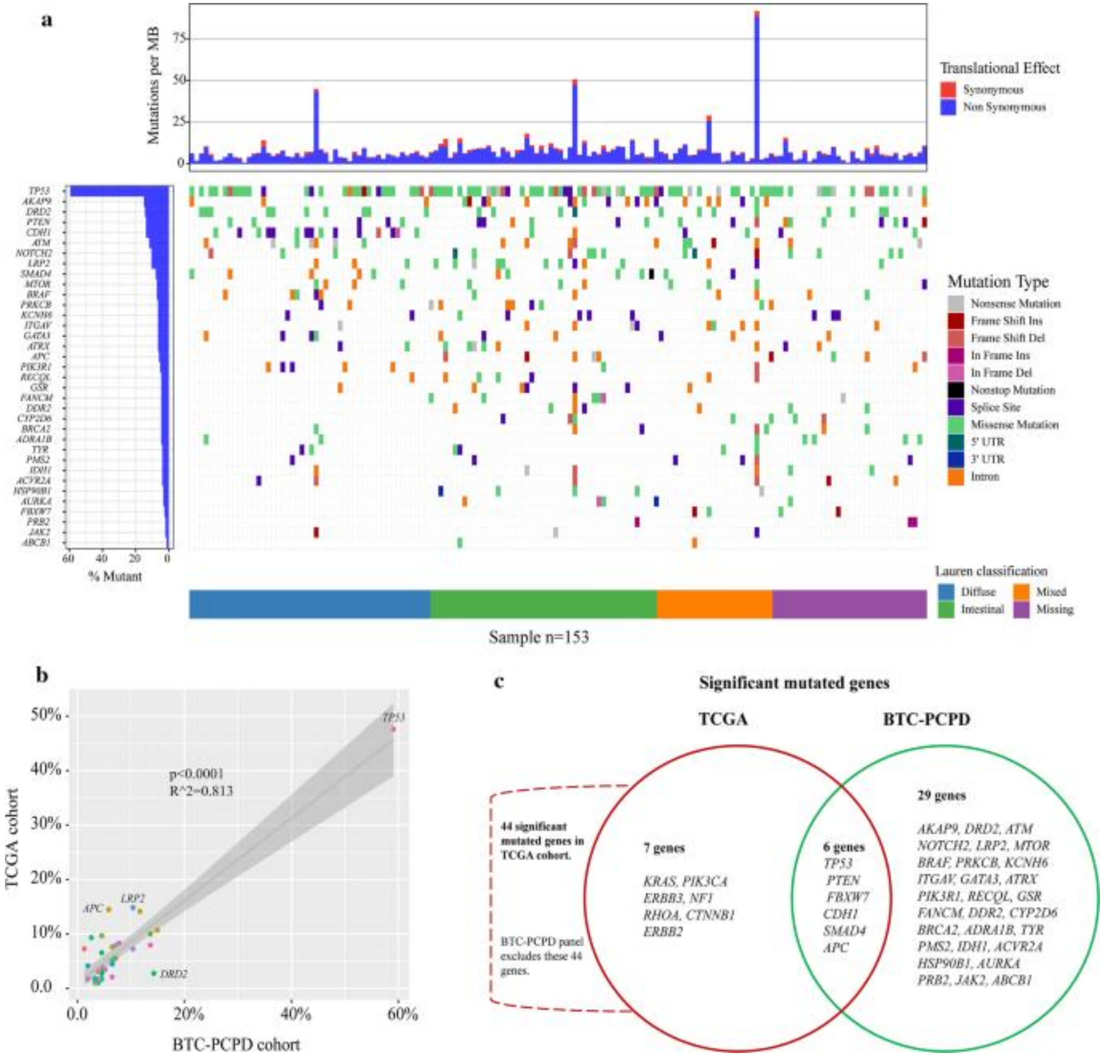
***Table 1:* Clinical criteria, recommended screening, and inherited alterations of familial gastric cancer syndromes**

tumor cell invasion

Oliveira C, et al. Lancet Oncol. 2015

Gene mutation profile of GC

- Capture-based NGS panel including 612 cancer-associated genes
- 153 gastric cancer patients
- 35 significantly mutated genes, such as *TP53*, *AKAP9*, *DRD2*, *PTEN*, *CDH1*, *LRP2 (novel)*
- Among them, 29 genes were novel significantly mutated genes compared with the TCGA study
- *TP53* was the most frequently mutated both cohorts. Correlations with male sex (p = 0.025) and tumor location in cardia (p = 0.011)



Cai et al. Journal of Translational Medicine. 2019

Novel gene mutations = biomarkers?

Top five most frequently mutated genes were *AKAP9* (14.94%), ***DRD2*** (14.29%), *ATM* (11.69%), *NOTCH2* (10.39%) *and* ***LRP2*** (10.39%).

DRD2 gene encodes the D2 subtype of the **dopamine receptor**.

Dopamine (DA), a neurotransmitter, has an important role in **tumor progression**.

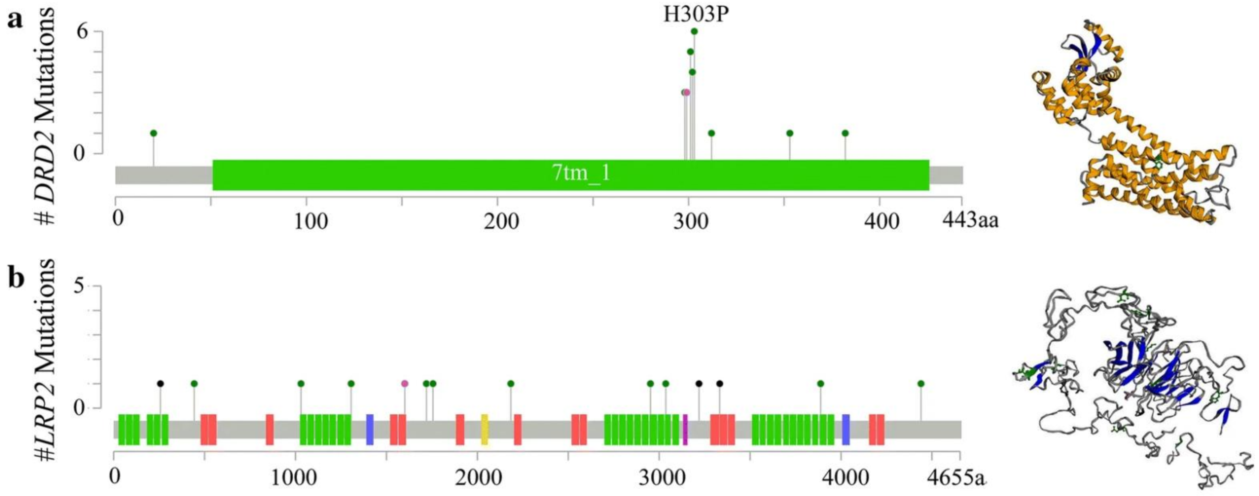
Previous studies indicated prominence of DR signaling in human cancer development and progression.

Dopamine D2 receptor regulates invasion and migration of GC cells via inhibition of the EGFR/AKT/MMP-13 pathway.

High expression of *DRD2* is correlated with poor prognosis of GC.

Basu S, et al. Endocrine. 2000
Huang H, et al. Int Immunopharmacol. 2016
Mu J, et al. Oncol Lett. 2017

LDL receptor-related proteins (LRPs) are receptors involved in **endocytosis, cell-signaling, and trafficking** of other cellular proteins.
LRP2 was the only LRP for which **high levels of mRNA expression correlated with improved patient survival**.



The proportion of mutations and protein structure of **a** *DRD2* and *LRP2* (**b**)

Cai et al. Journal of Translational Medicine. 2019

Somatic copy number alterations in GC

20 genes were significantly affected by SCNA.

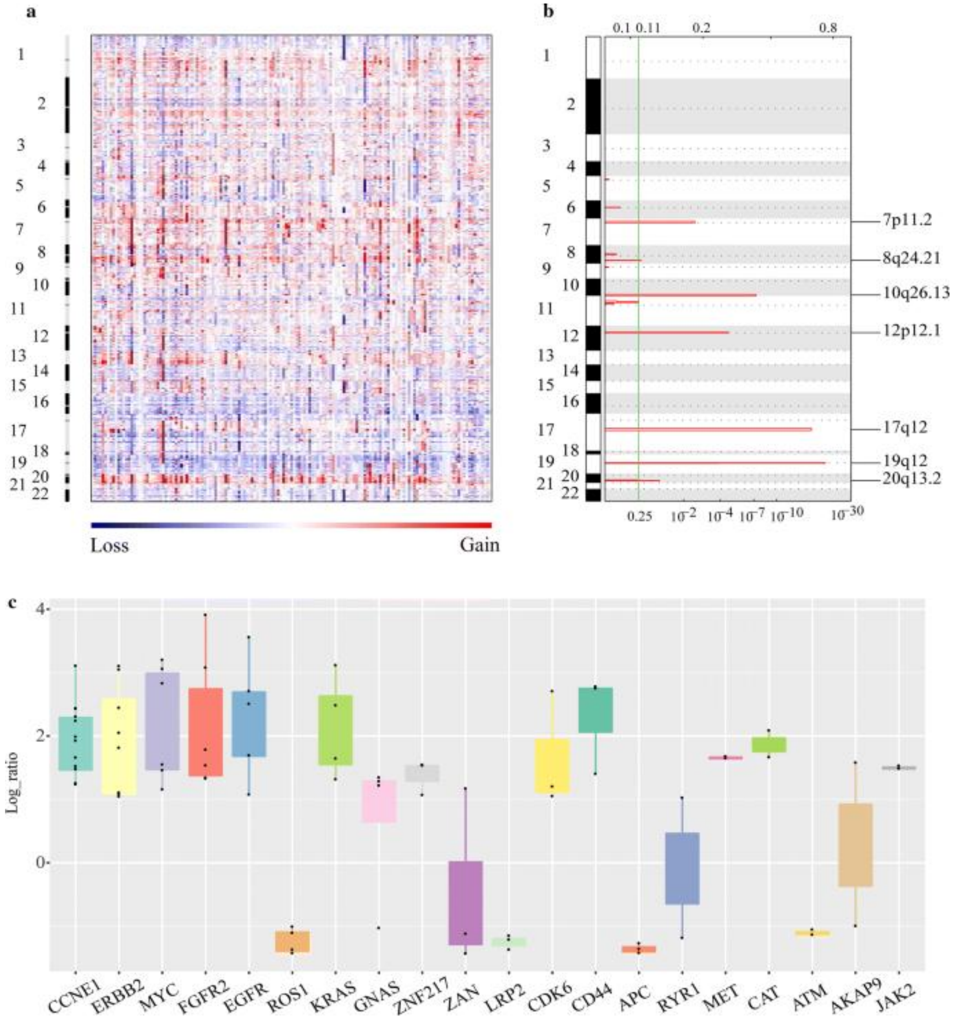
Amplifications were detected in 13 genes, while deletions were found in 5 genes. Both amplification and deletion were identified in the *RYR1* and ***AKAP9*** genes.

Amplifications mainly targeted well-known oncogenes such as *EGFR*, *ERBB2*, *KRAS*, *MYC*, *CCNE1*, *JAK2*, *FGFR2*.

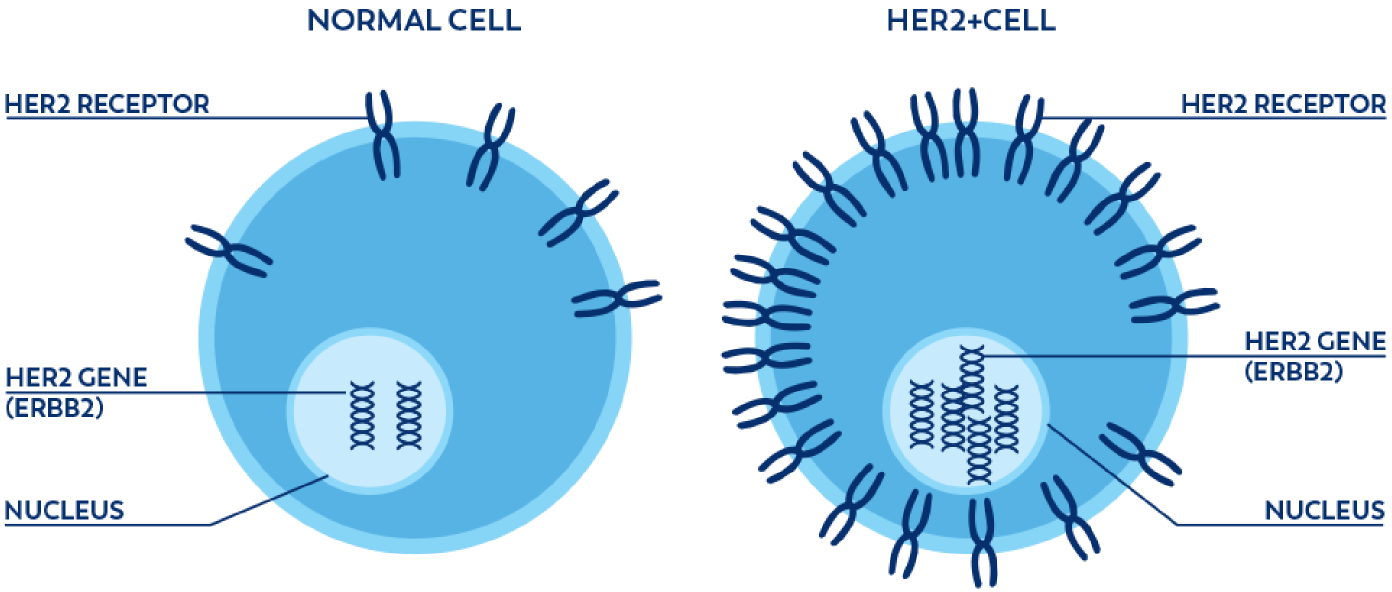
12 GC tissues harbored *CCNE1* amplifications (7.84%), a frequency similar to the TCGA cohort (10.58%). Amplification of *CCNE1* is associated with **poor outcome**.

Additionally, we also found amplification of *CD44*, which **is a gastric stem cell marker**.

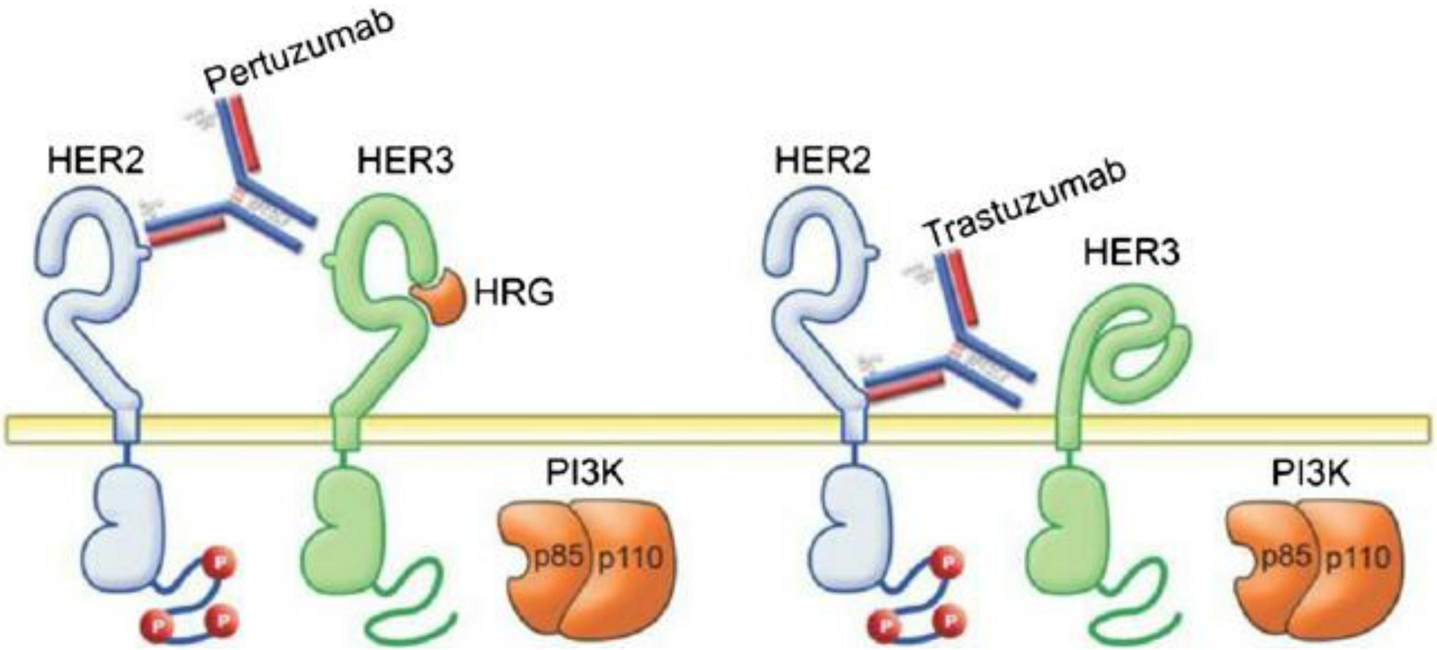
HER2 amplification was detected in 8 gastric tumor tissues (5.23%). These cases **respond well to trastuzumab & pertuzumab** (targeted therapies).



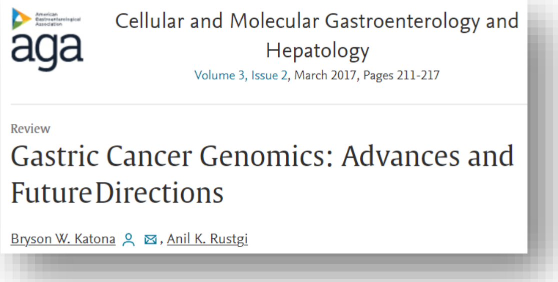
Cai et al. Journal of Translational Medicine. 2019



AMPLIFICATION – MULTIPLE HER2 GENES
OVEREXPRESSION – MANY HER2 RECEPTORS



Mutation enrichment in GC



Cellular and Molecular Gastroenterology and Hepatology
Volume 3, Issue 2, March 2017, Pages 211-217

Review

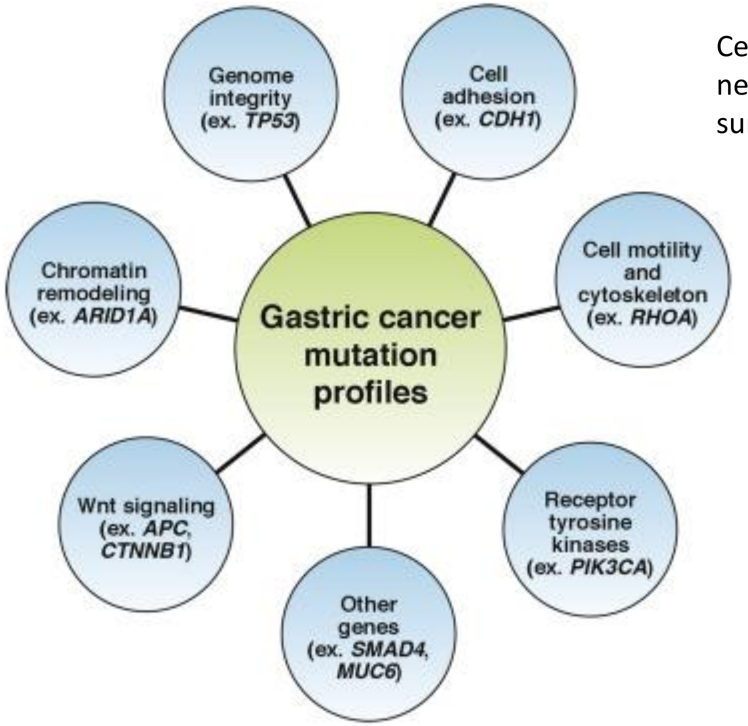
Gastric Cancer Genomics: Advances and Future Directions

Bryson W. Katona, Anil K. Rustgi

The maintenance of genome integrity is **essential for organism survival and for the inheritance of traits to offspring**. Genomic instability is caused by DNA damage, aberrant DNA replication or uncoordinated cell division, which can lead to chromosomal aberrations and gene mutations.

Chromatin remodeling genes, whose products are responsible for regulating chromatin structure to alter DNA accessibility and transcriptional efficiency, frequently are mutated in gastric cancer.

The Wnt signaling pathway is an ancient and evolutionarily conserved pathway that regulates crucial aspects of cell fate determination, cell migration, cell polarity, neural patterning and organogenesis during embryonic development.



Cell adhesion is the process by which cells interact and attach to neighbouring cells through specialised molecules of the cell surface.

The cytoskeleton is a structure that **helps cells maintain their shape and internal organization**, and it also provides mechanical support that enables cells to carry out essential functions like division and movement.

Receptor tyrosine kinases (RTKs) are the **high-affinity cell surface receptors** for many polypeptide growth factors, cytokines, and hormones.

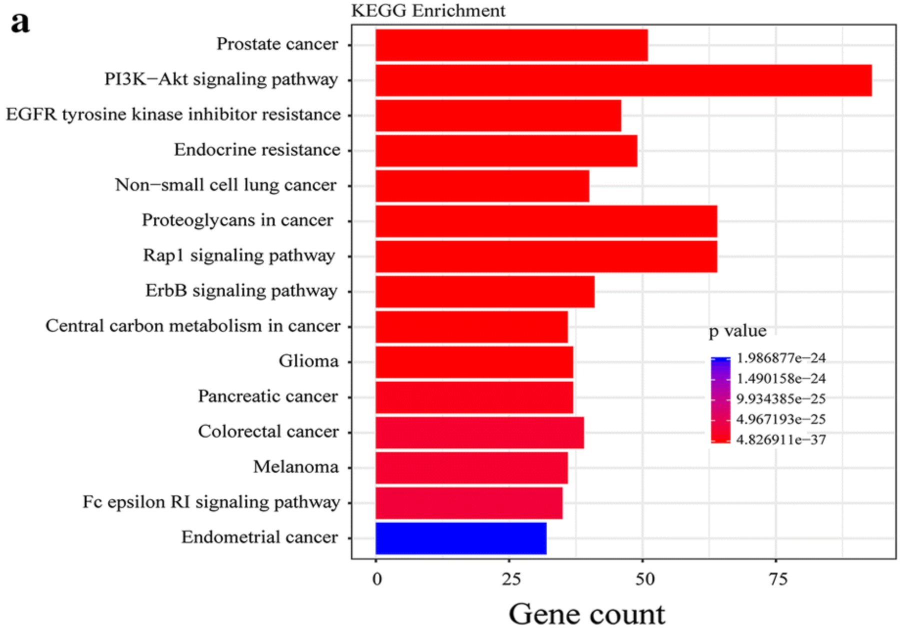
SMAD4 (8%) is involved in the transforming growth factor-β signaling pathway.
MUC6 (6%) is important for the production of cytoprotective mucin, in which inactivation may increase the risk of mucosal injury and subsequent carcinogenesis.

Mutation enrichment in GC

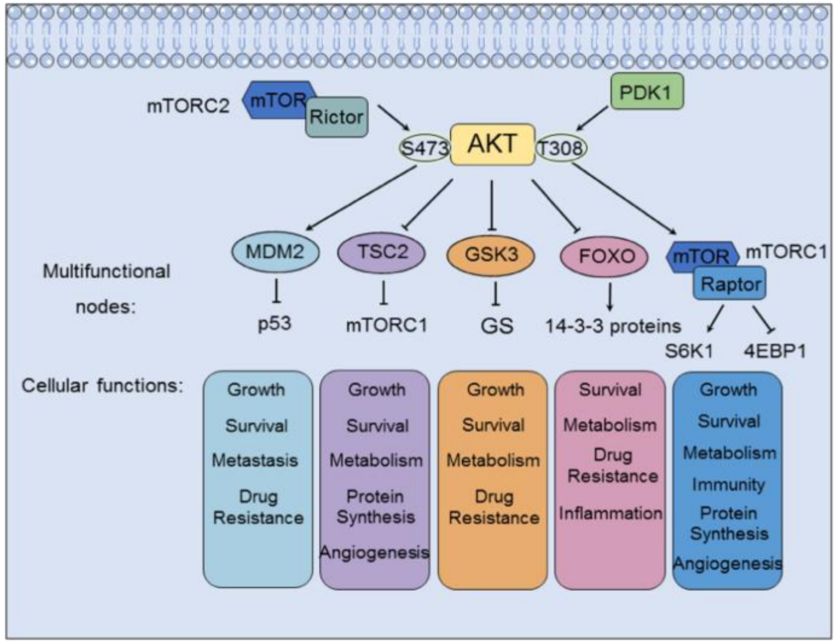
PI3K-Akt signaling pathway is **recurrently activated in different types of cancer**.

93 genes with somatic mutations were implicated in this pathway (~1/3 of all genes in the pathway).

13.07% patients harbored mutations in the *PTEN* gene, which is a **suppressor of the PI3K–Akt pathway**.



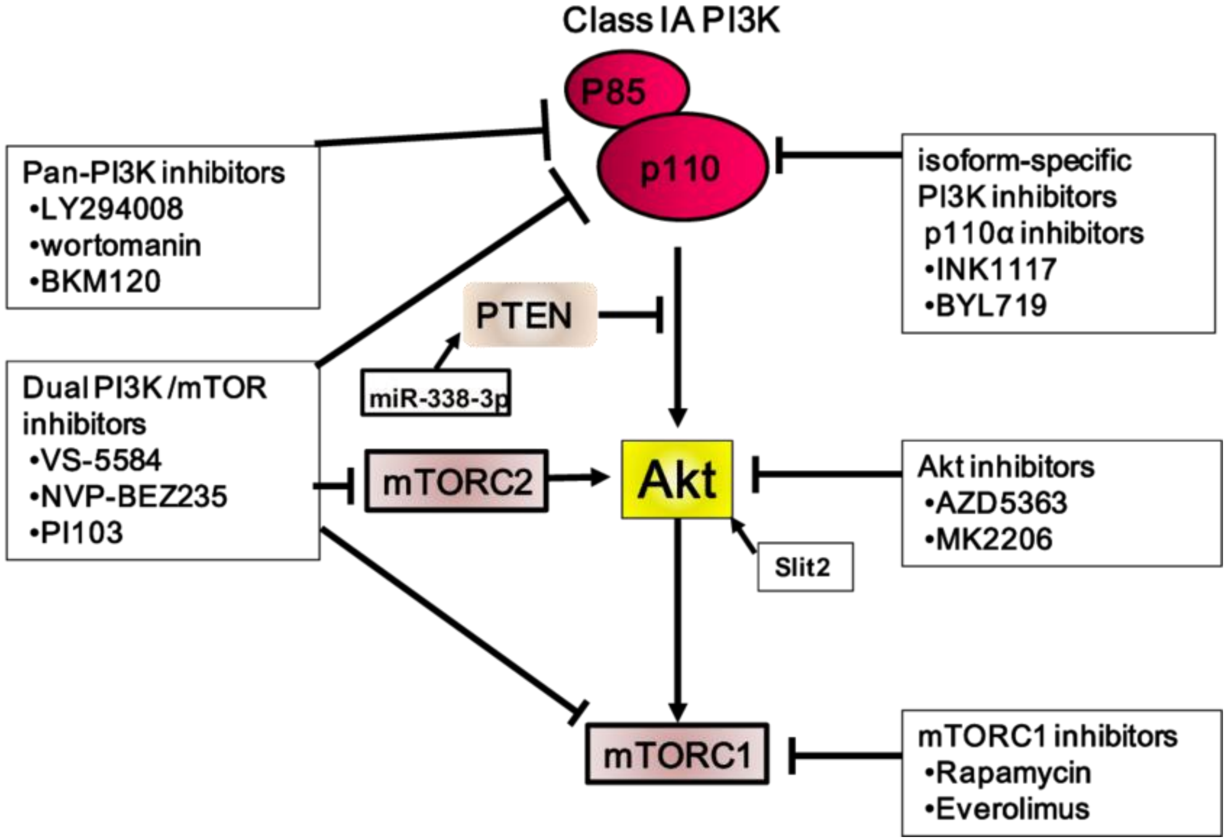
Cai et al. Journal of Translational Medicine. 2019



He et al. Signal Transduction and Targeted Therapy. 2021

PI3K-Akt Targeted therapies

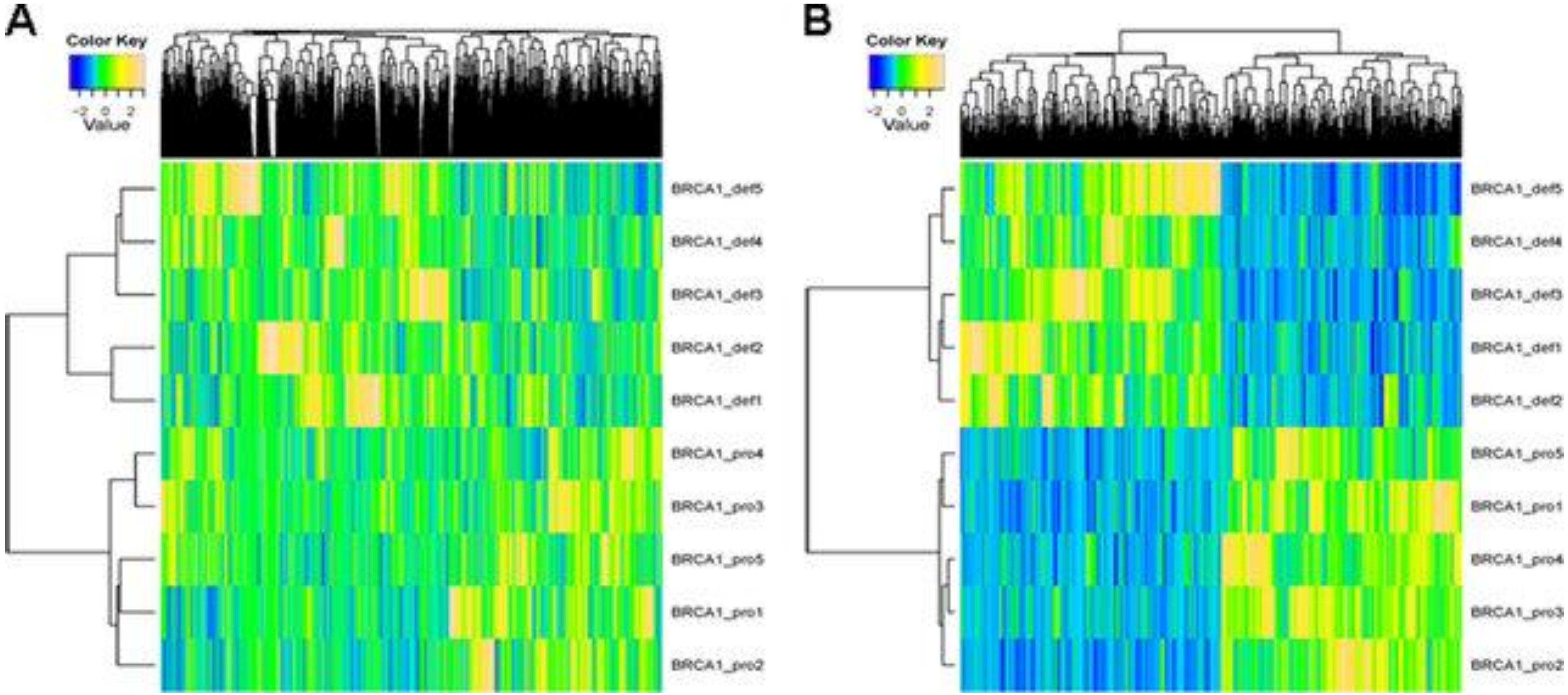
Development of both single, as well as recently, dual inhibitors essential for molecular targeted therapy for GC, (pan-class I inhibitors, isoform specific PI3K inhibitors, Akt inhibitors, dual Akt/mTOR inhibitors).



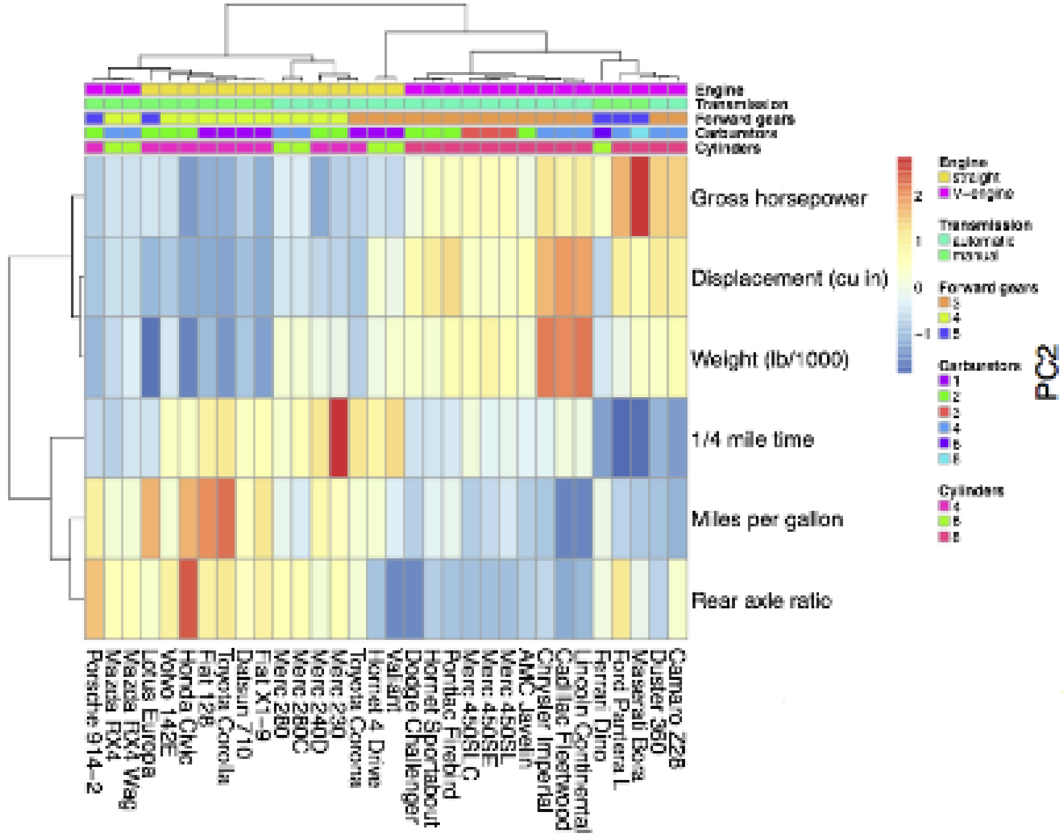
Matsuoka et al. Cancers 2014

Transcriptomics

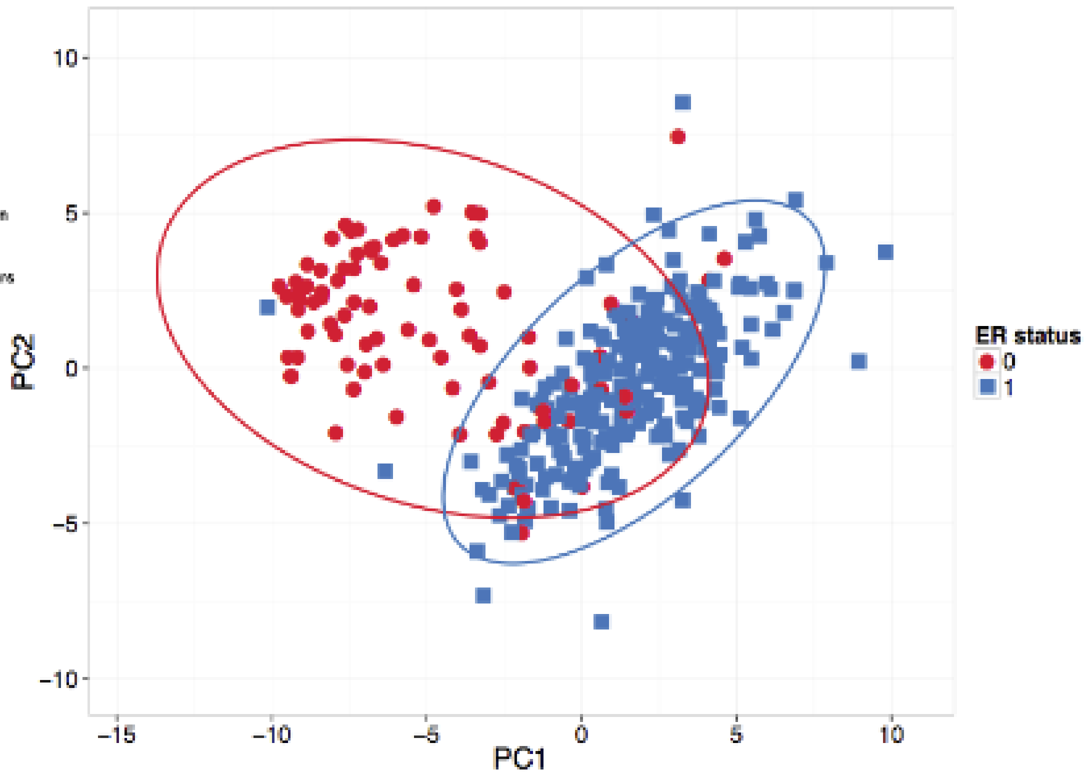
Unsupervised versus Supervised



Transcriptomic analysis



ClustVis



Transcriptomic analysis of GC

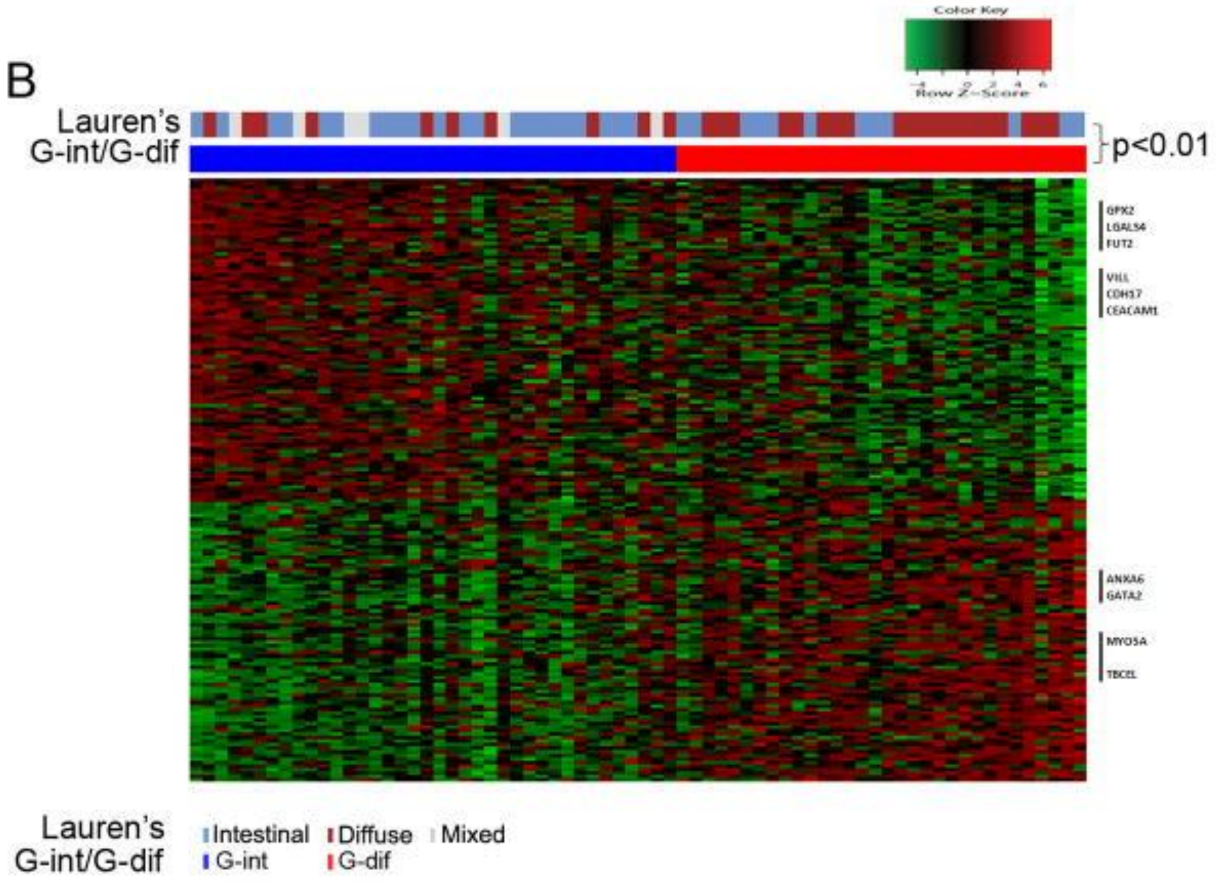
Gene expression profiles for 37 GC cell lines to identify intrinsic GC subtypes. Validation in primary tumors from 521 patients.

Associations of intrinsic transcriptomic subtypes with Lauren’s classification in primary GCs.

171 gene set can robustly classify primary tumors into G-INT and G-DIF sub-classes.

G-INT subtype: **carbohydrate and protein metabolism** (*FUT2*) and **cell adhesion** (*LGALS4, CDH17*)

G-DIF subtype: **cell proliferation** (*AURKB*) and **fatty acid metabolism** (*ELOVL5*)

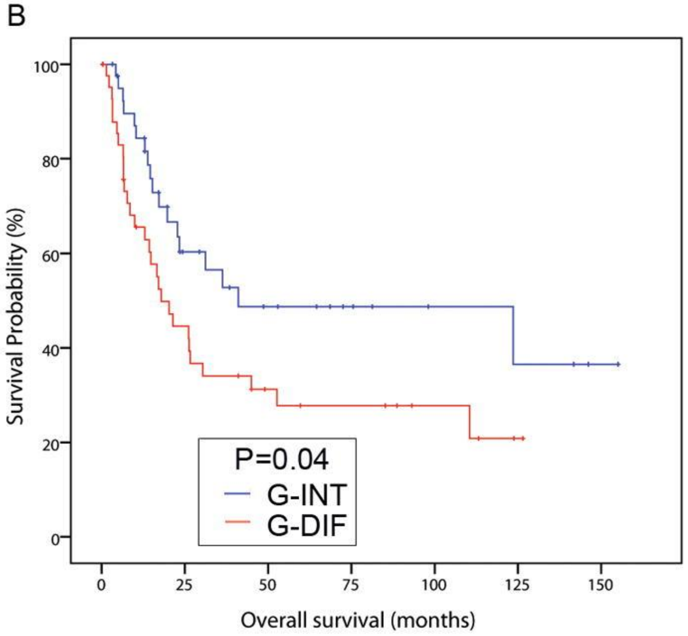


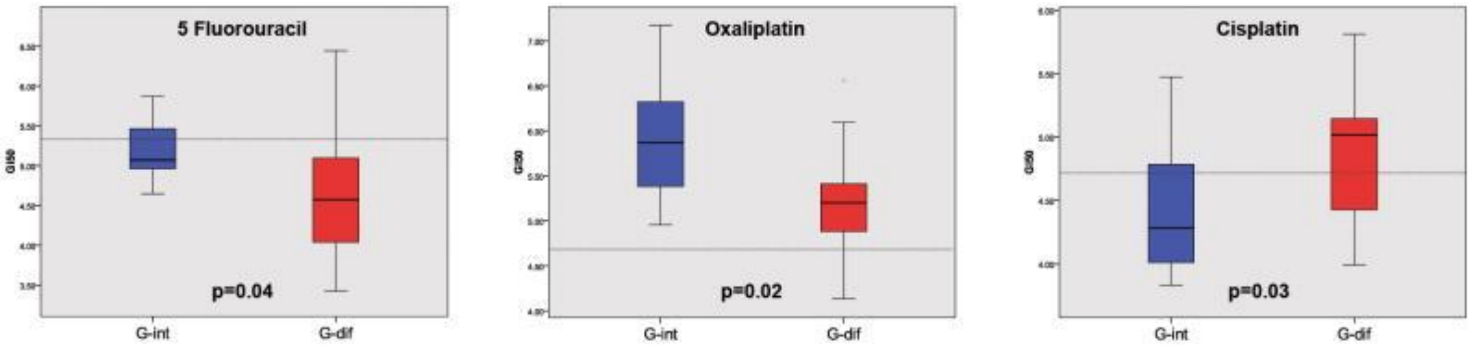
Tan IB, et al. Gastroenterology. 2011;141:476-485.

Clinical effect

Prognostic value

Predictive value





In vitro chemosensitivity of G-INT and G-DIF cell lines

Even when the intrinsic classification and Lauren's classes are discordant

Tan IB, et al. Gastroenterology. 2011;141:476-485.

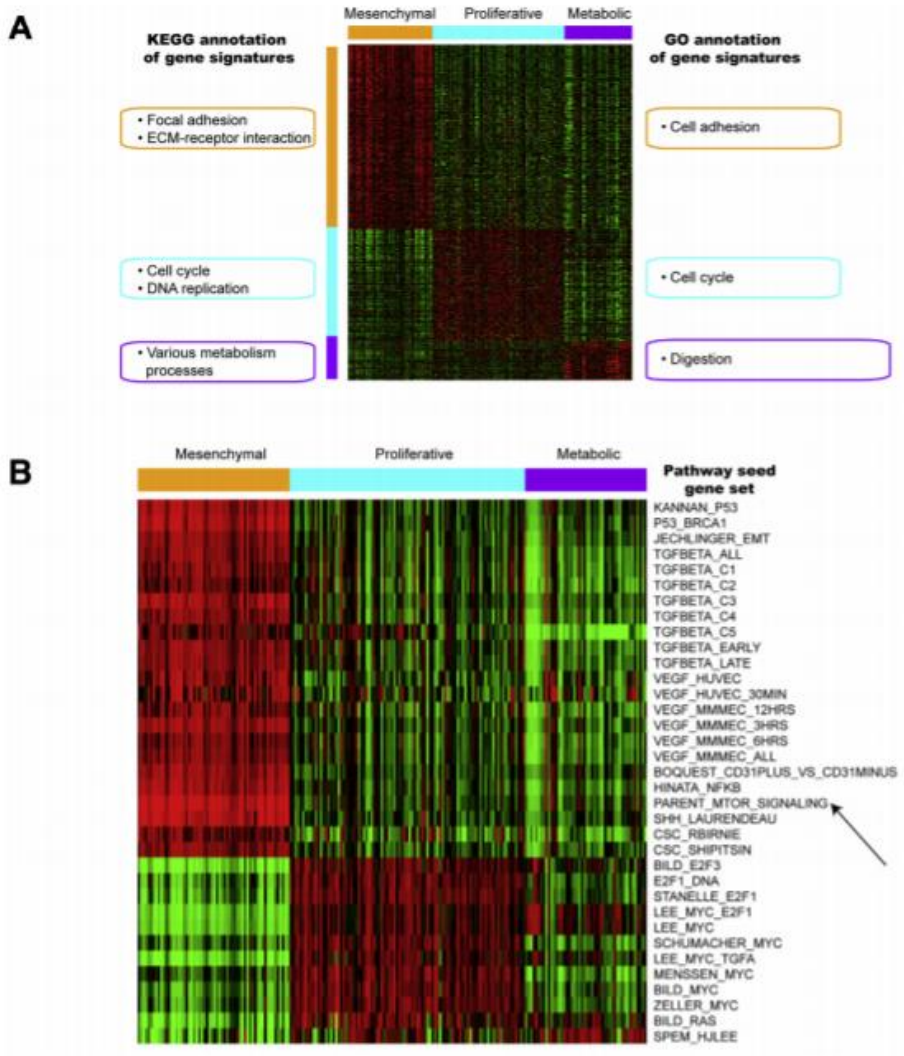
Transcriptomic analysis of GC

Gene expression patterns among **248 gastric tumors**.

3 subtypes of gastric adenocarcinoma: **proliferative**, **metabolic**, and **mesenchymal**.

The mesenchymal subtype is strongly associated with the Lauren diffuse type,

whereas the proliferative subtype is strongly associated with the intestinal type.



Lei Z, et al. Gastroenterology. 2013

Molecular characterization

Biological level

The proliferative subtype is characterized by more frequent *TP53* mutations - is enriched significantly for high-CNA tumors.

The mesenchymal subtype is enriched for low-CNA tumors - has cancer stem cell–like properties: (i) activation of relevant pathways, (ii) poorly differentiated gastric cancers , (iii) similar hypermethylated CpG sites.

Clinical level

Metabolic subtype: greater benefits with 5-fluorouracil treatment.

Mesenchymal subtype: particularly sensitive to phosphatidylinositol 3-kinase/AKT/mTOR inhibitors *in vitro*.