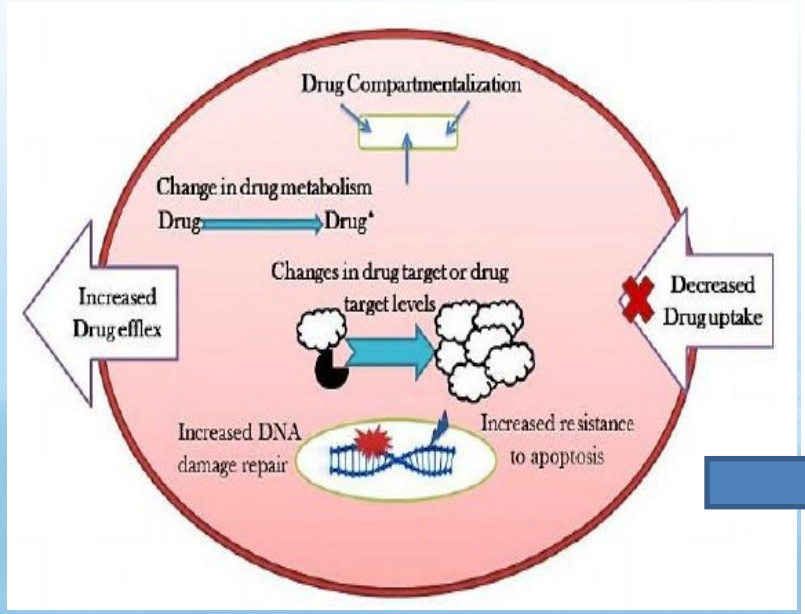
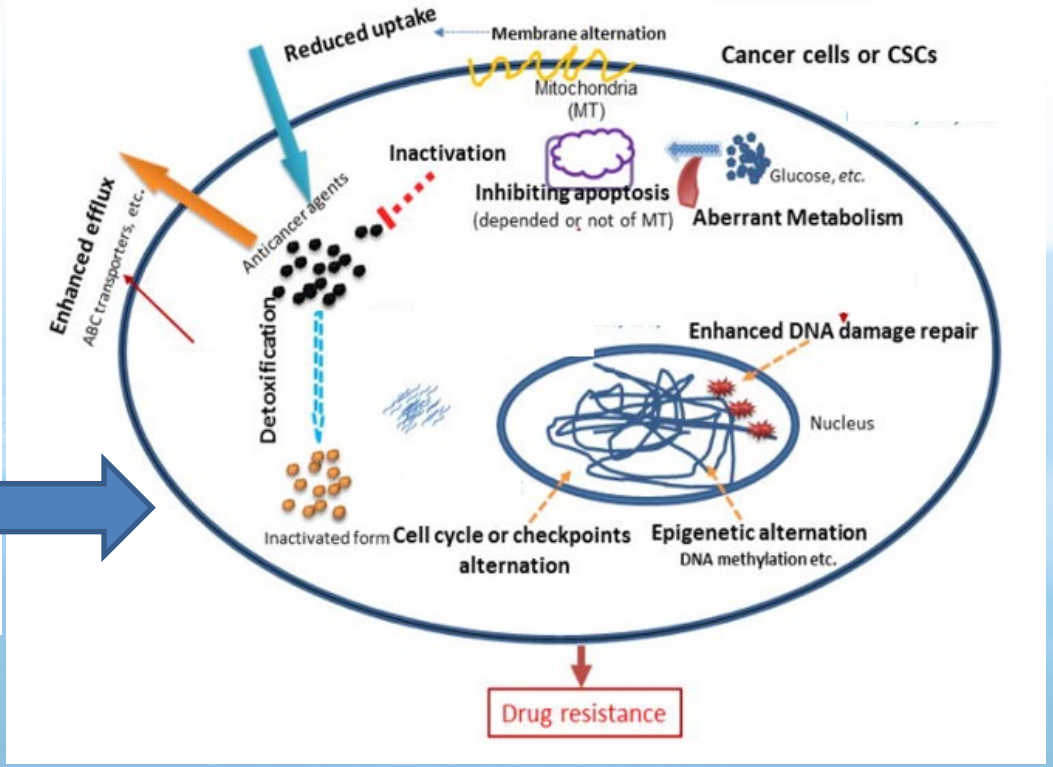
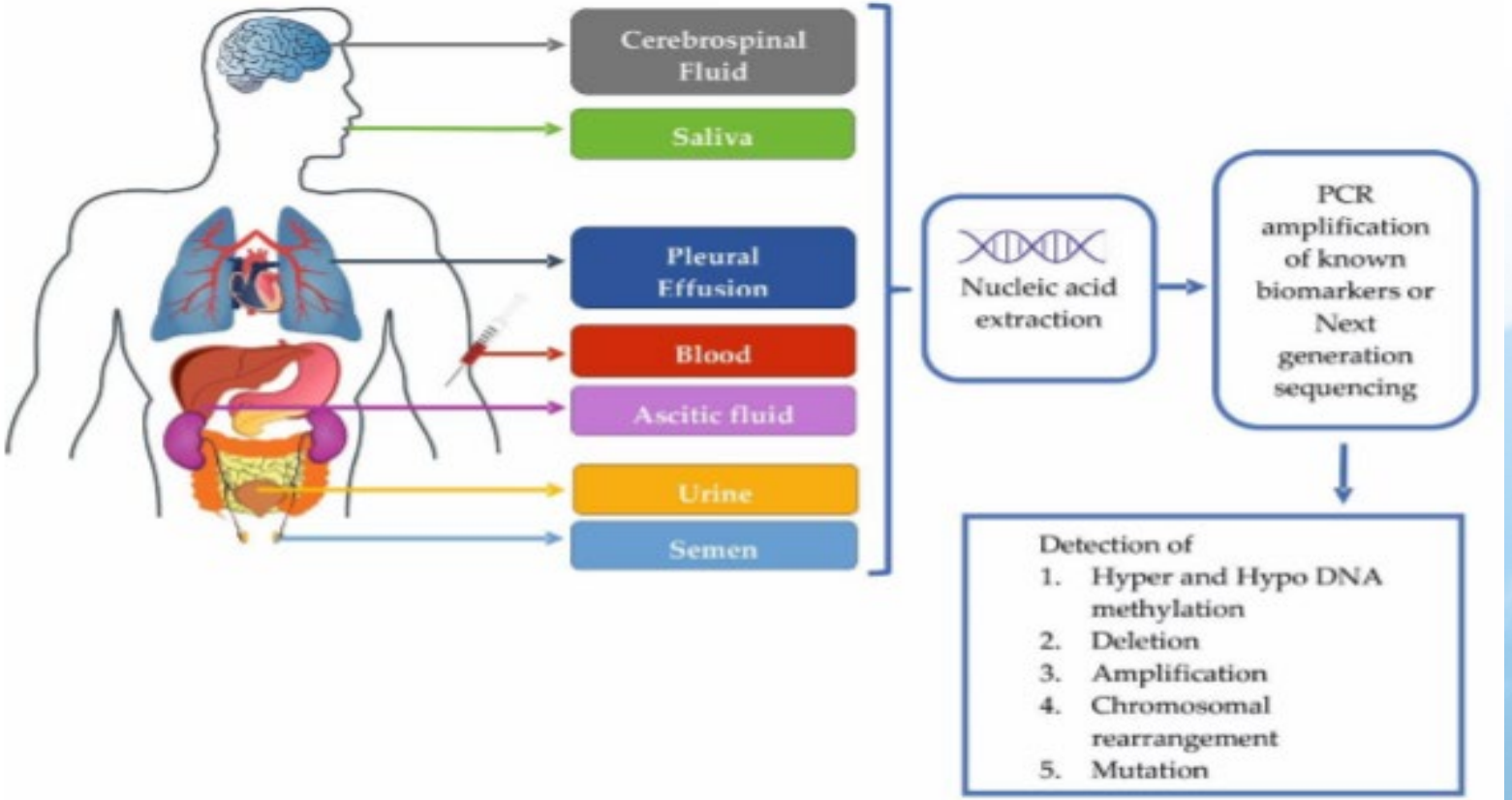
Mechanisms of Immune Checkpoint Inhibitor

Circumstances	Mechanism	Circumstances	Mechanism
PD-L1–overexpressed tumors	Nonreversible and severe T-cell exhaustion	Association with neoantigen overexpression by genetic alterations in mammalian SWI/SNF chromatin remodeling complexes	Loss-of-function mutations in chromatin remodeler genes (PBRM1, ARID2 and BRD7) sensitize tumors to ICB and increase accessibility to regulatory elements of IFN-γ–inducible genes.
	Coexpression of inhibitory receptors (LAG-3, TIM-3, TIGIT, VISTA, and BTLA)		Loss of ARID1A leads to increased MSI with inability to recruit mismatch repair genes during DNA repair, increasing mutational burden and neoantigen load.
	Decreased ratio of TILs to Tregs and MDSCs		Stability of chromatin remodeling complexes in tumors contributes to ICB resistance
	Altered metabolism through IDO & increased adenosine production	High mutation overload tumors	Decreased antigen presentation secondary to MHC, β2-microglobulin, and NLRC5 alterations
	Mutations in PTEN, EGFR, and MYC		AK1/2 mutations and decreased IFN-γ signaling
PD-1 and CTLA-4 inhibitor combination therapy	Immunoediting with loss of neoantigens		Upregulation of alternate inhibitory checkpoints
	Deletions or mutations in JAK1/2, IFNGR1/2, & IRF1		
	Decreased T-cell priming and DC dysfunction		
	Aberrant WNT/β-catenin signaling		
	High copy number loss of tumor suppressor genes		

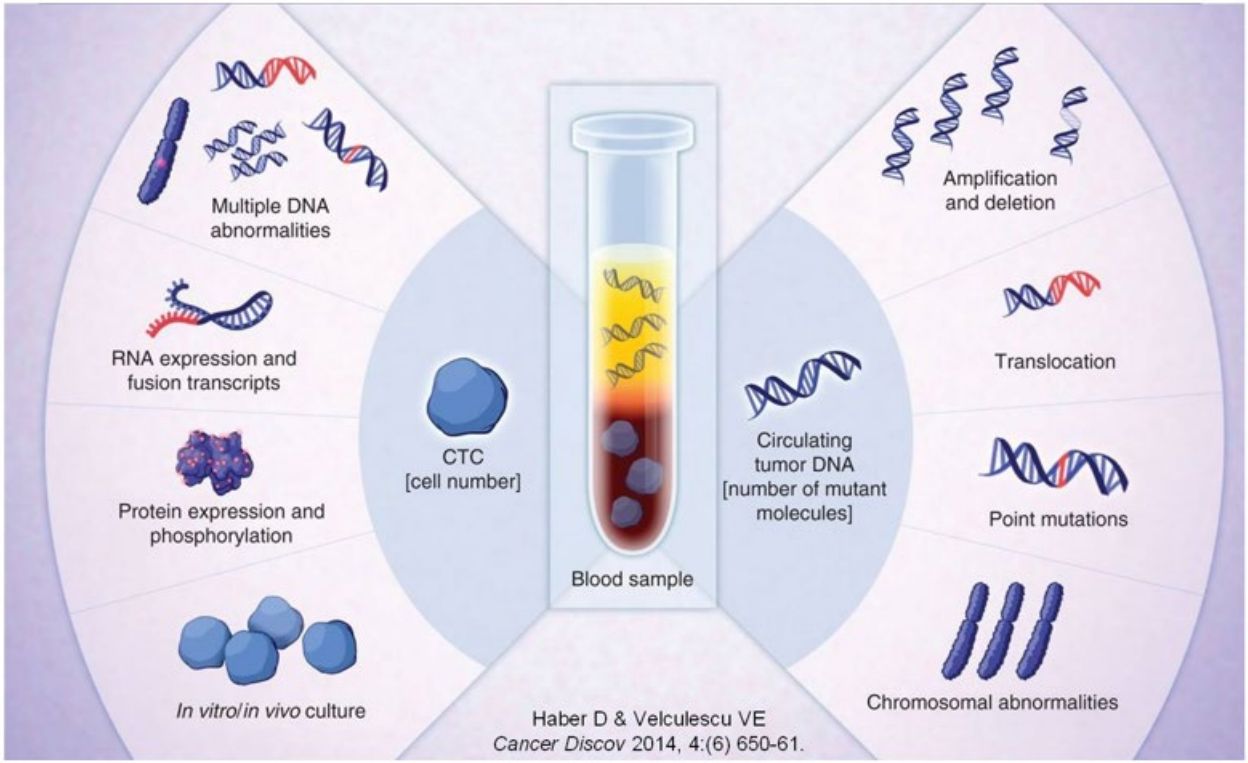
Mechanism of Drug Resistance





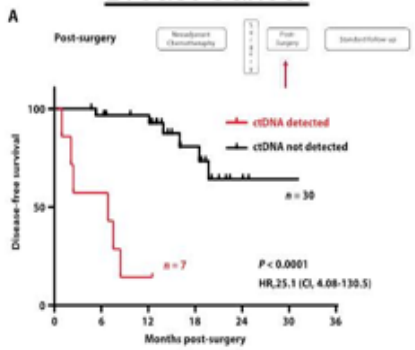

Cerebrospinal Fluid
Saliva
Pleural Effusion
Blood
Ascitic fluid
Urine
Semen
Nucleic acid extraction
PCR amplification of known biomarkers or Next generation sequencing
Detection of
1. Hyper and Hypo DNA methylation
2. Deletion
3. Amplification
4. Chromosomal rearrangement
5. Mutation

Liquid Biopsies: CTCs & ctDNA

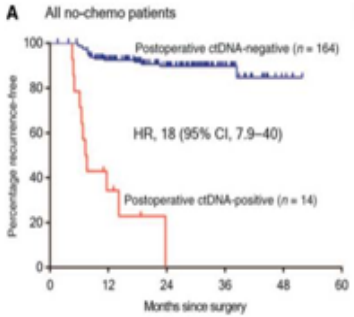


- Minimal residual disease (MRD):
  - Small volume of tumor cells remaining after treatment in patients who have no clinical evidence of disease
- No reliable MRD assays exist for most solid tumors
- ctDNA MRD detection has recently been demonstrated in breast and colon cancer using personalized assays

Breast Cancer



Colon Cancer

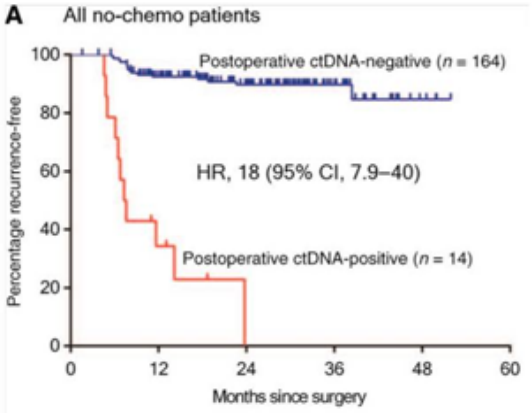


ctDNA for MRD in Colon Cancer

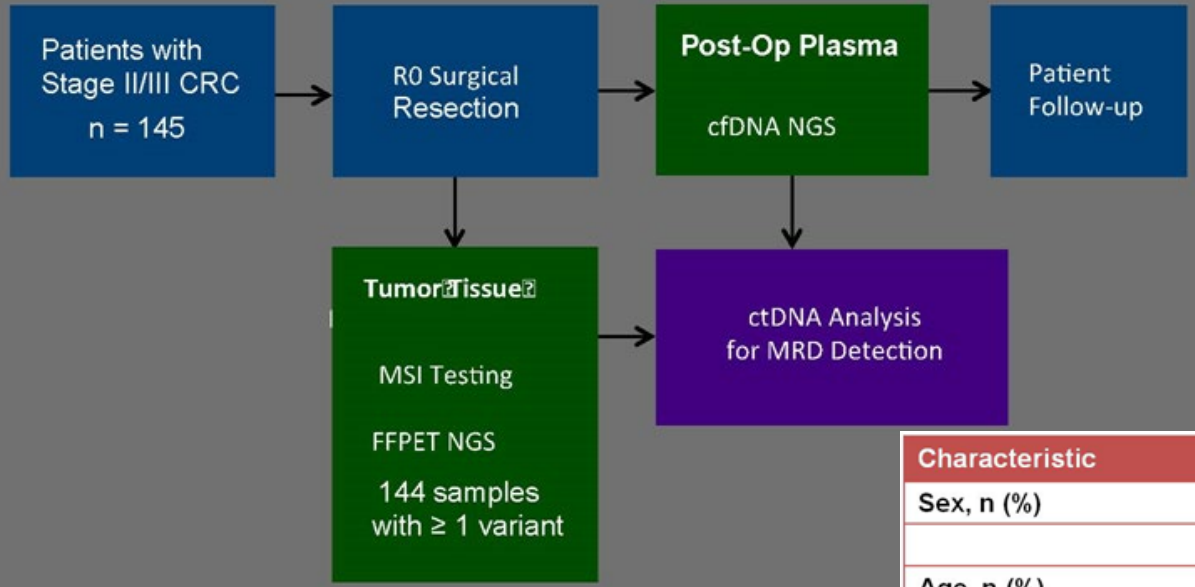
- Recurrence after resection of colon cancer thought to mainly arise from occult micro-metastases
- Resection alone cures ~80% of stage II and ~50% of stage III CRC[1]
- In US, most stage II colon cancer patients do not receive adjuvant chemotherapy while most stage III patients do
- Ability to detect MRD might allow personalization of adjuvant therapy in both stage II and III colon cancer

ctDNA for MRD in Colon Cancer

- Tie et al analyzed ctDNA MRD in 231 stage II colon cancer patients (178 not treated with adjuvant chemo)
- Sequenced tumors and designed personalized, single mutation NGS assays (Safe-SeqS)
- Analyzed plasma samples drawn 4-10 weeks post-op



Recurrence prediction:
-Sensitivity = 41%
-Specificity = 98%
-PPV = 79%
-NPV = 90%



Diehn, Alizadeh et al. ASCO 2017

Characteristic		Number (n=144)
Sex, n (%)	Female	56 (39)
	Male	88 (61)
Age, n (%)	< 70	50 (35)
	> 70	94 (65)
Follow-up, median	(mo.)	31.2
Stage, n (%)	II	85 (59)
	III	59 (41)
Tumor Type	Colon	93 (65)
	Rectum	51 (35)
MSI by PCR, n (%)	MSI-H	31 (22)
	MSI-L	113 (78)
Adjuvant chemotherapy, n (%)	No	110 (76)
	Yes	34 (24)

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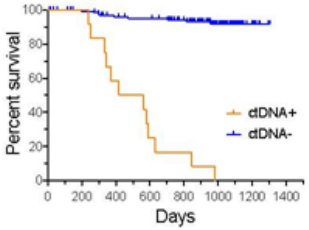
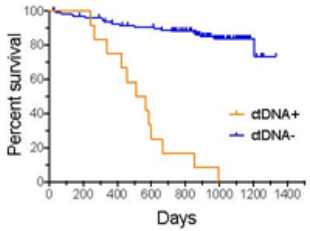
A TTR



HR **27.8**
95% CI: 11.3-68.7
p<0.0001

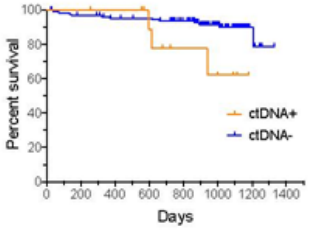
B RFS



HR **12.1**
95% CI: 5.7-25.9
p<0.0001

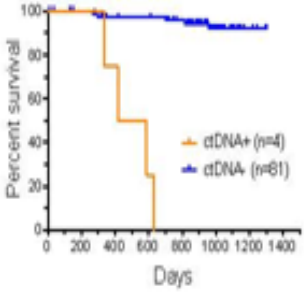
2 year RFS: 17% vs 88%

C OS



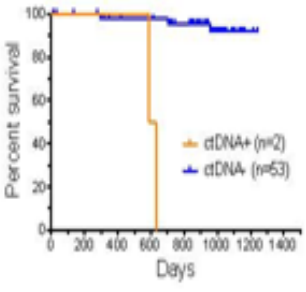
HR **3.6**
95% CI: 1.0-13.1
p=0.0342

A TTR: Stage 2 All



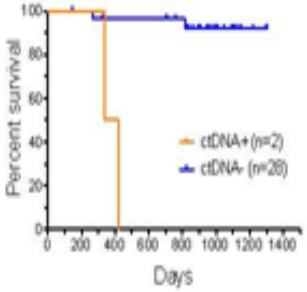
HR **54.4**
95% CI: 9.5-311.7
p<0.0001

B TTR: Stage 2 Colon



HR **57.4**
95% CI: 5.1-649.2
p<0.0001

C TTR: Stage 2 Rectum



HR **32.8**
95% CI: 2.9-371.7
p<0.0001

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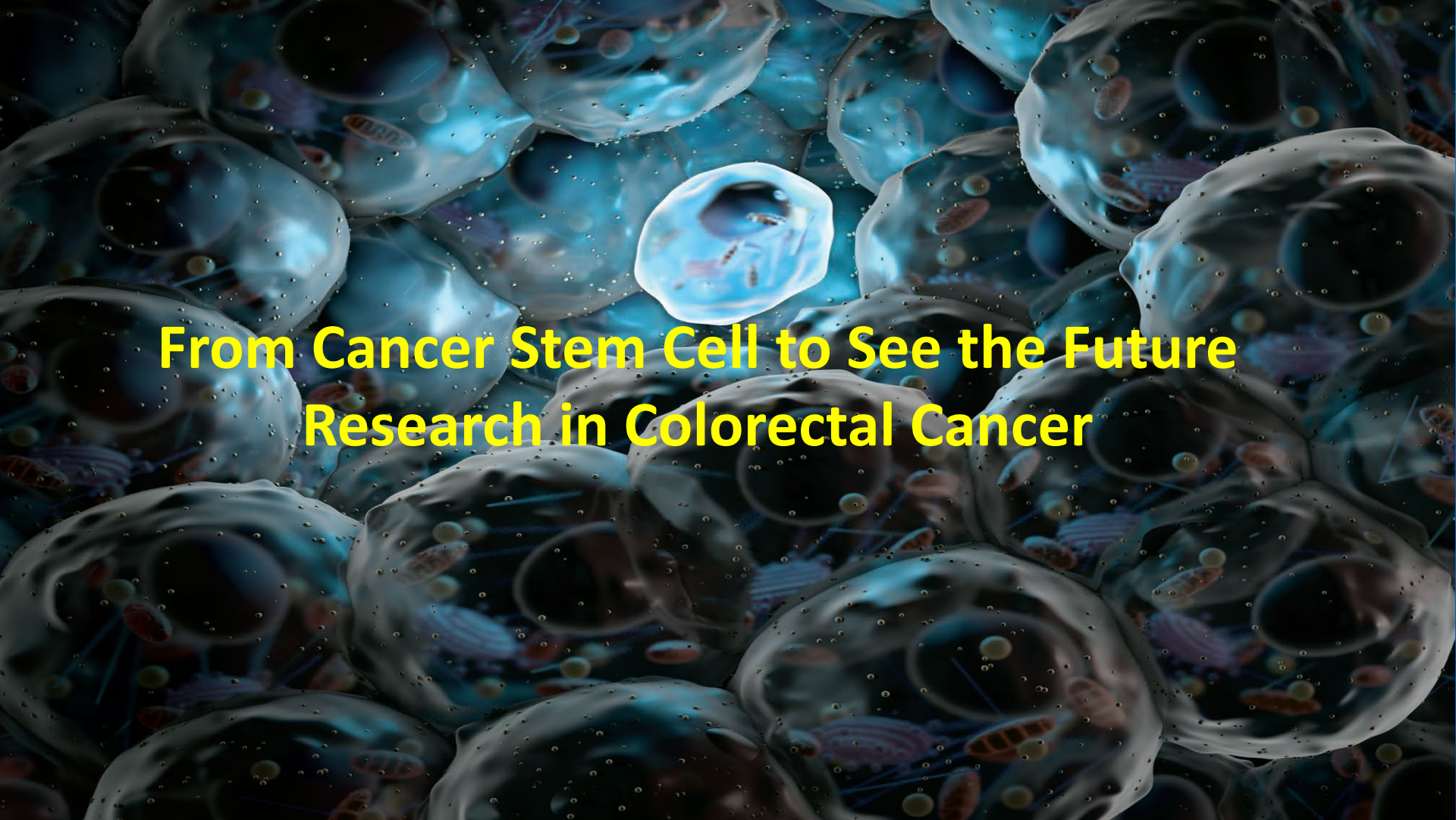
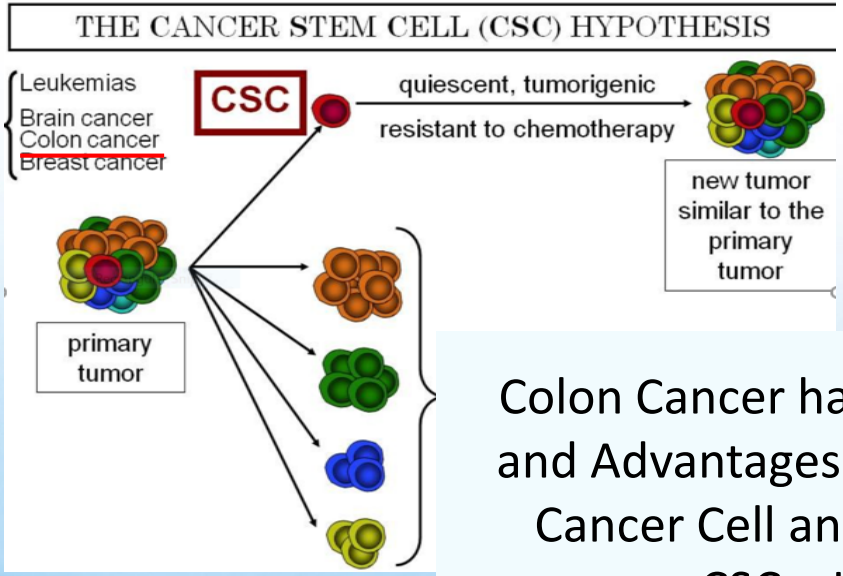
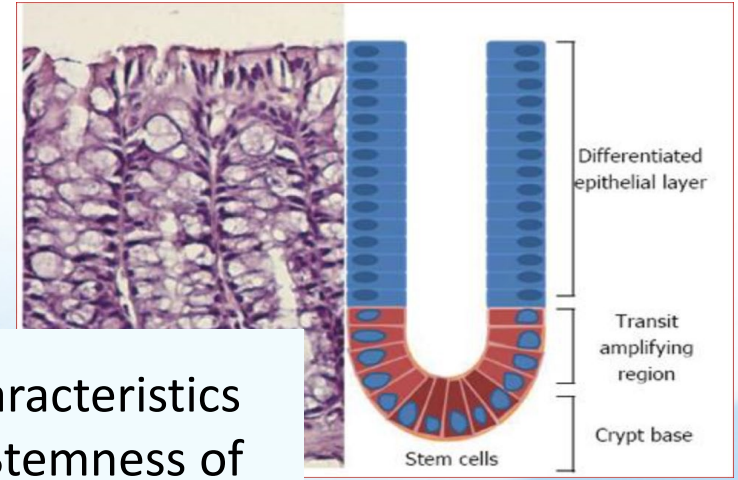
Factors associated with TTR

Variable	All Patients (n=144*)			Stage II Colon, no adjuvant (n=51)		
	HR	95% CI	p value	HR	95% CI	p value
Age, < 70 vs ≥ 70	1.33	0.56 - 3.15	0.522	2.23	0.37 - 13.38	0.379
Sex, female vs male	0.81	0.33 - 2.02	0.655	0.75	0.13 - 4.52	0.758
T stage T3 vs T4	0.82	0.24 - 2.80	0.752	N/A		0.997
Lymph node yield, < 12 vs ≥ 12	N/A		0.992	N/A		0.997
Tumor differentiation, poor vs well/moderate	1.12	0.43 - 2.89	0.815	0.67	0.07 - 5.96	0.716
Lymphovascular invasion, no vs yes	0.61	0.25 - 1.52	0.293	0.91	0.15 - 5.49	0.921
PNI, - vs +	0.45	0.16 - 1.28	0.135	N/A		0.997
MSS or MSI-L vs MSI-H	1.66	0.49 - 5.64	0.416	1.32	0.22 - 7.92	0.760
Postoperative ctDNA status, + vs -	27.81	11.26 - 68.67	**<0.0001**	52.29	4.63 - 590.97	**0.0014**
High vs Low Cliincopathologic Risk	1.27	0.53 - 3.07	0.594	1.82	0.30 - 10.88	0.514

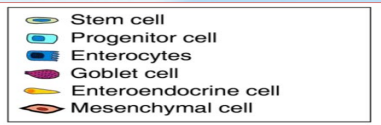

From Cancer Stem Cell to See the Future
Research in Colorectal Cancer

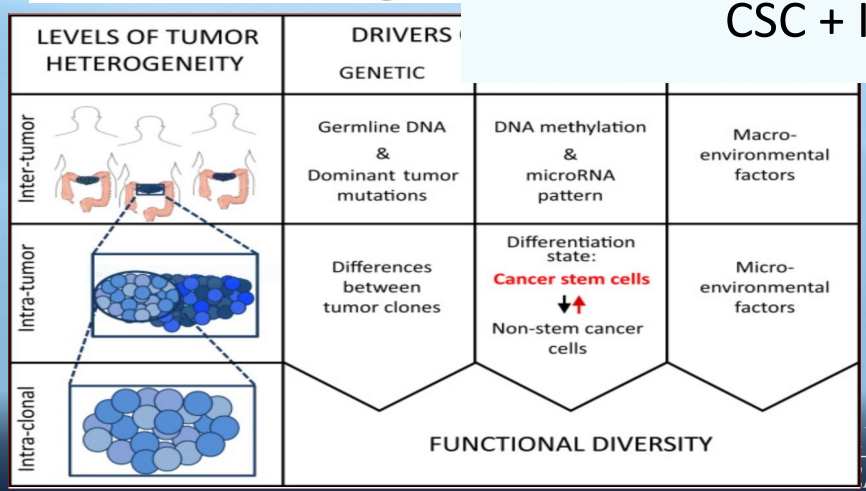


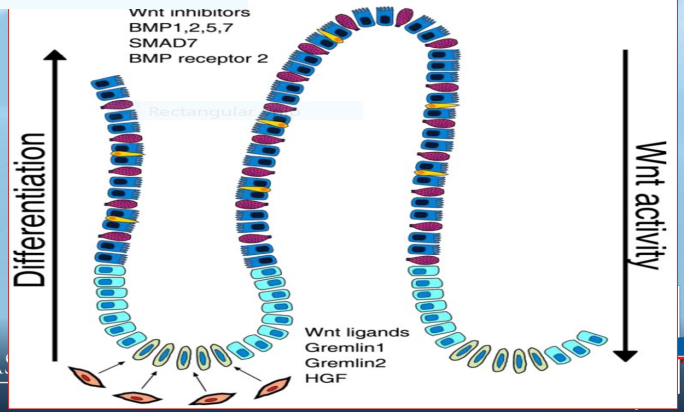


Colon Cancer has the Characteristics and Advantages of both Stemness of Cancer Cell and Intestinal System

CSC + ISC → **CCSC**





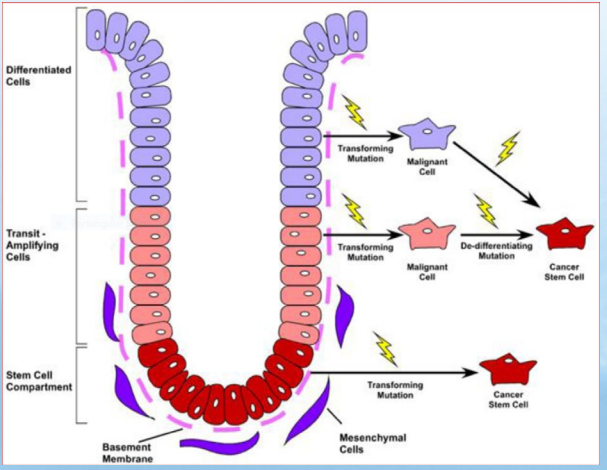


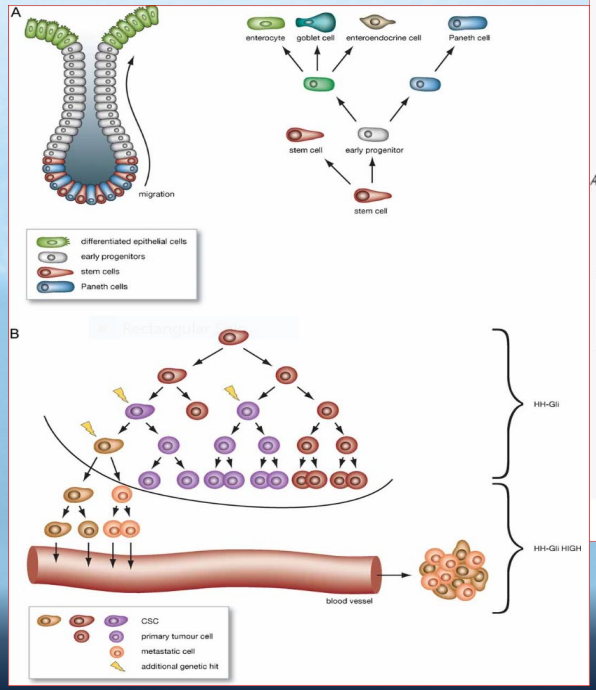
Cancer Stem Cell

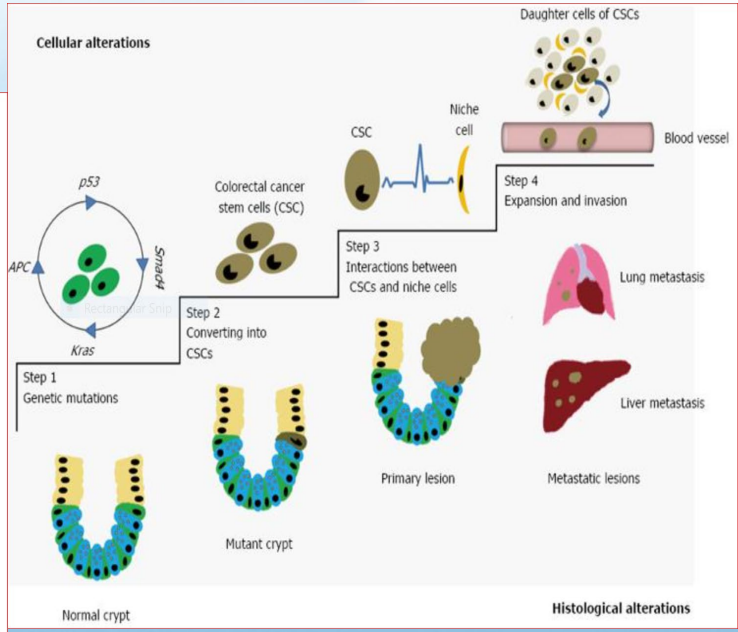
- Possess special biological properties
  - Long-term self-renewal capacity
  - Multi-lineage differentiation
- **Resistance** to conventional chemotherapy and radiotherapy
- A major source of residual disease after therapy →'**Recurrence**'
- Identified in blood, breast, brain, and colon cancer
- Wnt, Hedgehog and Notch pathways are involved

- Very Important to understand the cellular survival mechanisms
  - To eradicate cancer stem cells and preventing chemotherapy and radiotherapy resistance.
  - To develop effective therapeutic approaches
  - To eliminate CSC to improve the treatment outcome of cancer patients
  - To eradicate the main root cause of cancer

- CSCs represent phenotypically and functionally heterogenous populations
- Colorectal CSCs are dynamic populations (not a static)
- Populations continuously altered by various extrinsic factors and microenviroment in addition to intrinsic cellular factor

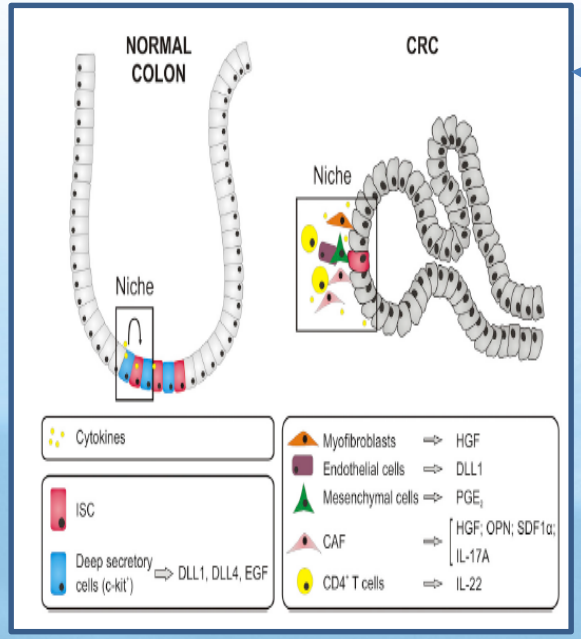


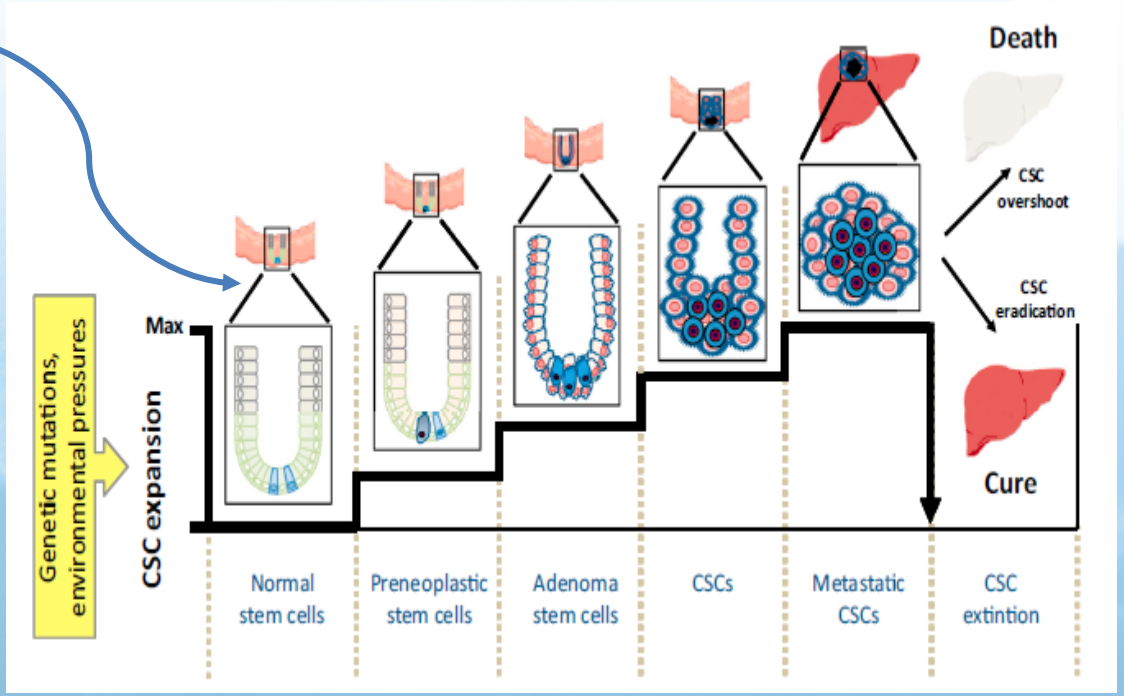




Tumor Evolutions

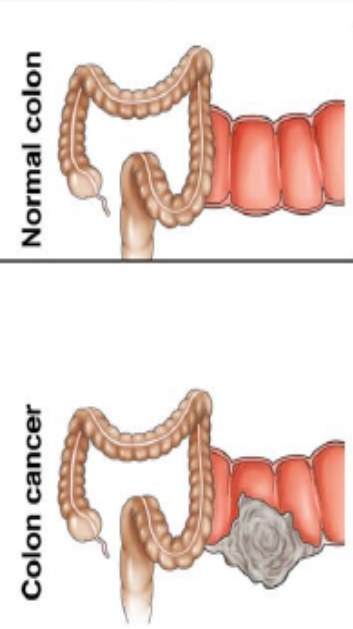
Zeuner A, et al Cell Stem Cell, 2014





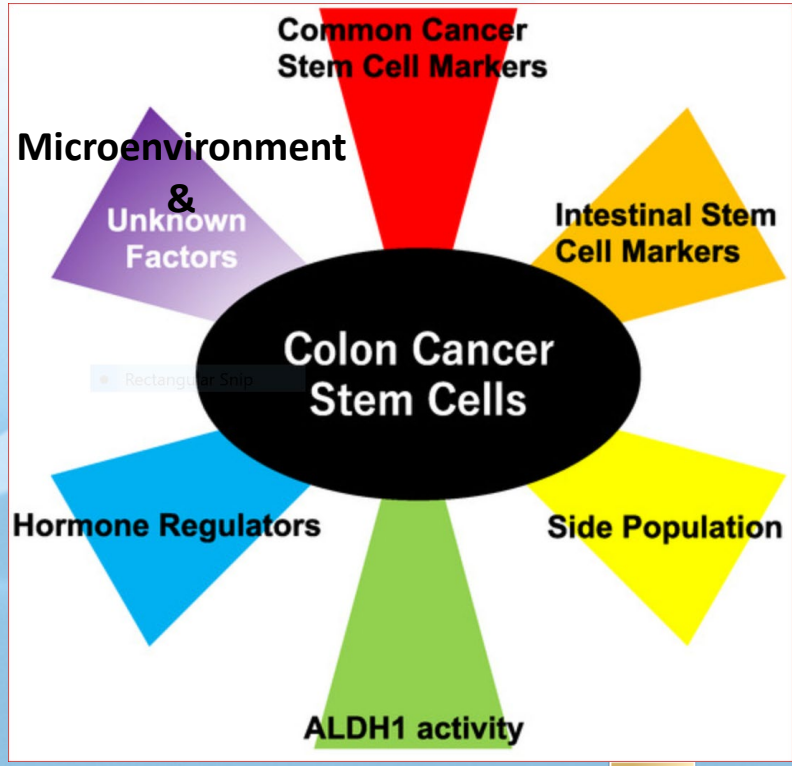
Intestinal Stem Cell and Cancer Stem Cell Markers

Todaro M et al, Gastroenterology 2010



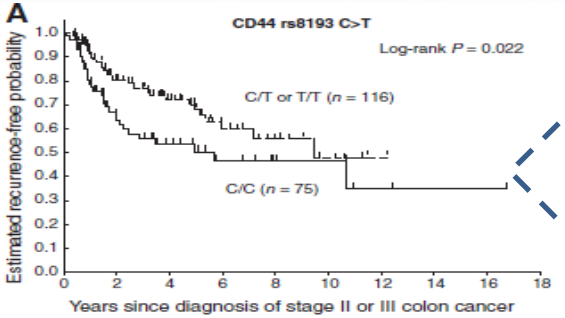
Marker	Other name	Function
Msi-1		RNA-binding protein
CD29	Integrin b1	Cell adhesion molecule
Lgr5	GPR49	Unknown, Wnt target gene
DCAMKL-1		Kinase
CD133	Prominin 1	Self-renewal, Tumor angiogenesis
ESA	EpCAM, BerEp4	Cell adhesion molecule
CD44	CDW44	Cell adhesion molecule, Hyaluronic acid receptor
CD166	ALCAM	Cell adhesion molecule
Msi-1		RNA-binding protein
CD29	Integrin b1	Cell adhesion molecule
CD24	HSA	Cell adhesion molecule
Lgr5	GPR49	Unknown, Wnt target gene
ALDH1	ALDC	Enzyme

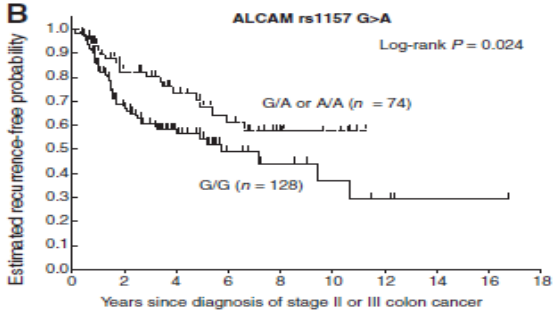
Complexity of CCSC

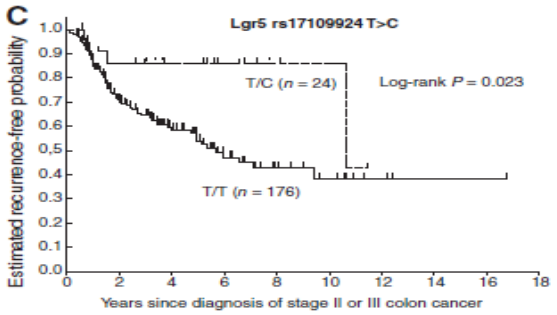


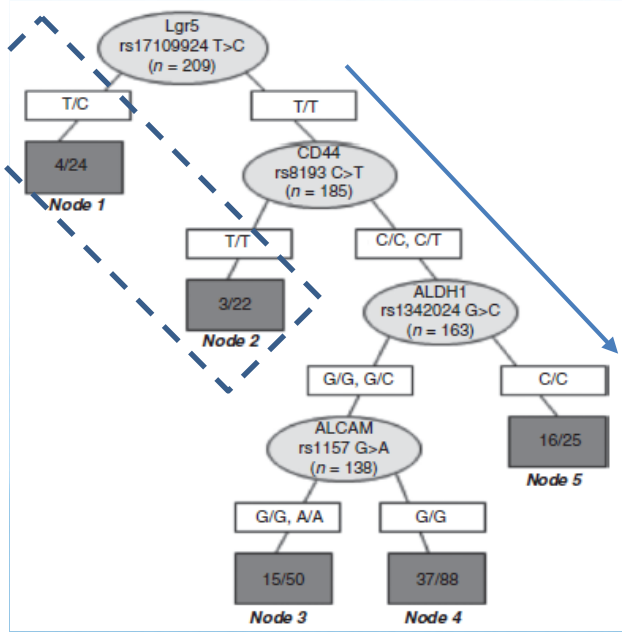
Association of CSC Markers and the Outcomes of Stage III Colon Cancer

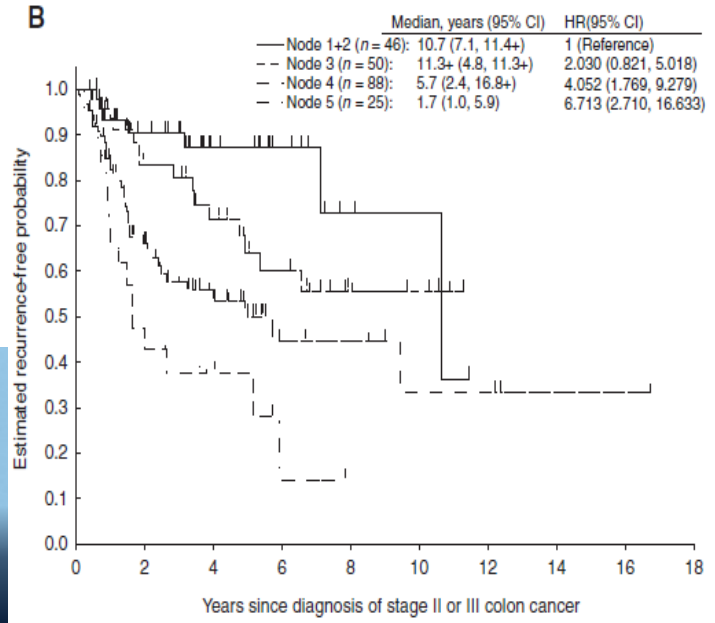
Gerger A et al Clin Cancer Res 2011







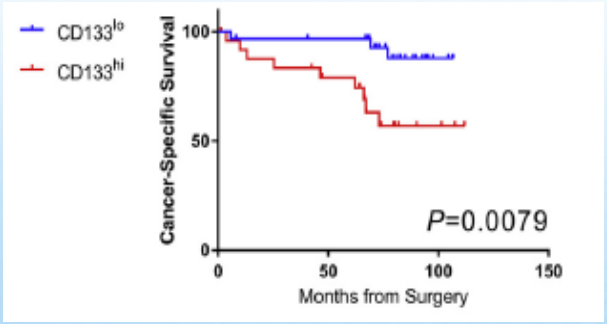


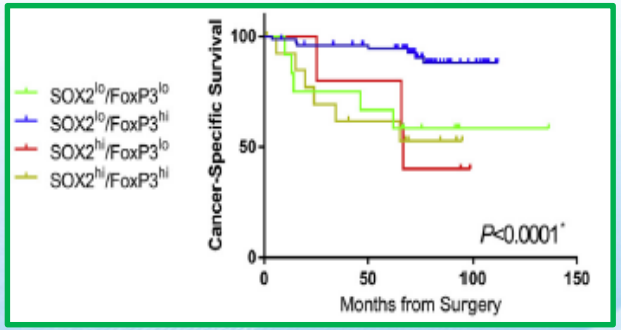


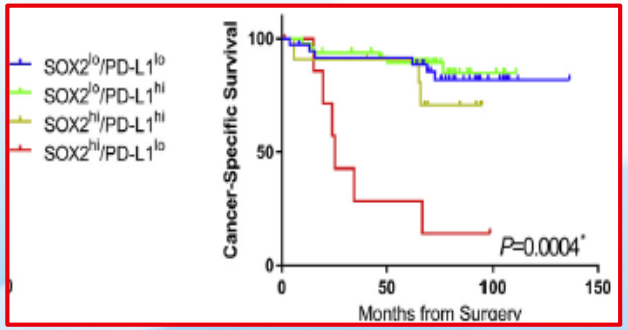
THE UNIVERSITY OF KANSAS CANCER CENTER

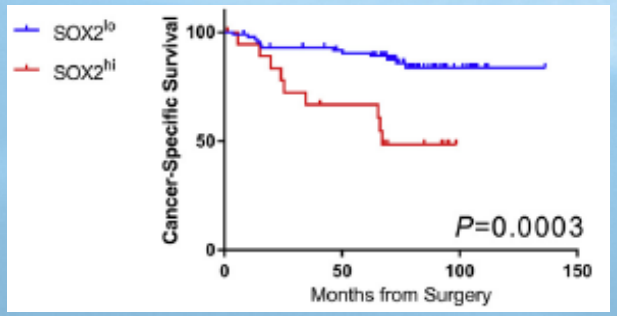
The Outcomes of Colon Cancer -- Associated of CSC Interaction & Microenvironment

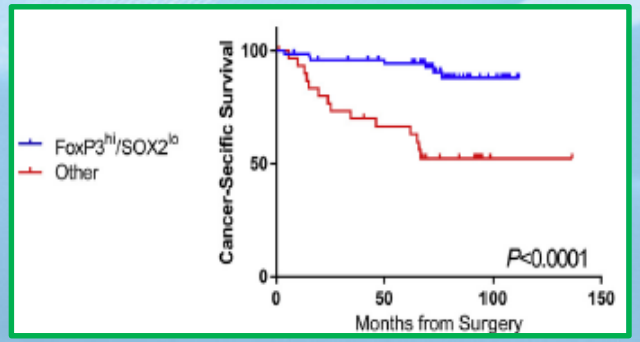
Miller TJ et al, Pathology 2017

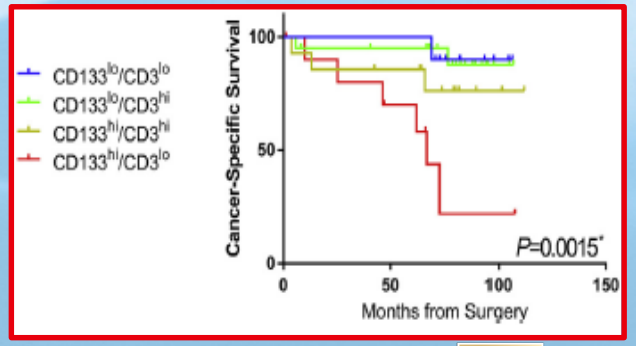












High-Fat Diet (HFD) Increases LGR5 Expression and Promotes Tumor Growth via STRA6 activation transduces a JAK2-STAT3 signaling cascade → Colon Carcinogenesis and Colon Cancer Self-renewal

Karunanithi S, et al Stem Cell Report 2017