Poster discussion Basic and Translational Research

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Disclosure slide

- no Conflicts of Interest to declare

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Optimizing gemcitabine efficacy through degradation of RRM1

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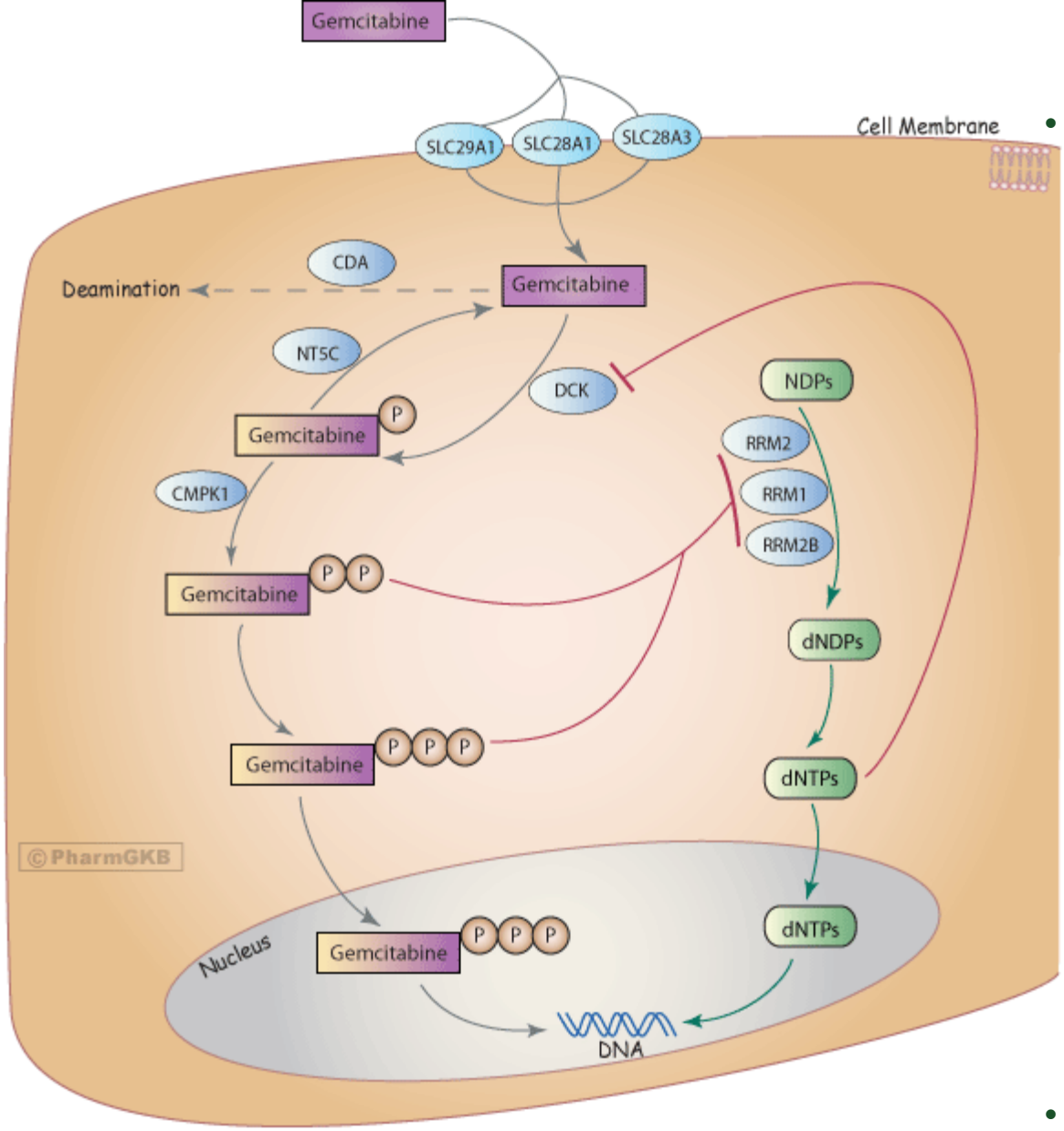
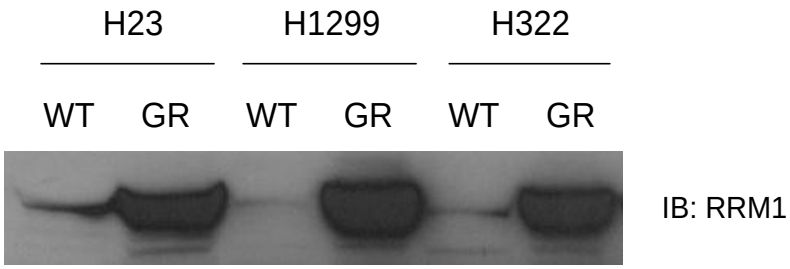


- Gemcitabine (2'2-difluorodeoxycytidine [dFdC]) is a specific analogue of the native nucleotide deoxycytidine.

- Ribonucleotide reductase (RR) is the rate-limiting step in DNA synthesis. It is the only known enzyme that converts ribonucleotides to deoxyribonucleoside for DNA polymerization and repair
- RR is a holoenzyme consisting of dimerized RR subunit 1 and 2 (RRM1, RRM2).
- RRM1 has been shown to function with the p53-regulated RRM2 homologue p53R2, which is important in DNA repair secondary to genotoxic stress

RRM1 (Ribonucleotide Reductase M1)

- RRM1 (ribonucleotide reductase M1) is the molecular target and key efficacy determinant of gemcitabine. Gemcitabine binds directly to active sites resulting in irreversible inactivation.
- High RRM1 level was associated with gemcitabine resistance (GR) in non-small cell lung cancer cells.



Upregulation of RRM1 protein level in gemcitabine resistant (GR) non-small cell lung cancer cell lines comparing to wild type (WT)

Cancer. 2012 May 1;118(9):2525-31.

Preliminary indication of survival benefit from ERCC1 and RRM1-tailored chemotherapy in patients with advanced nonsmall cell lung cancer

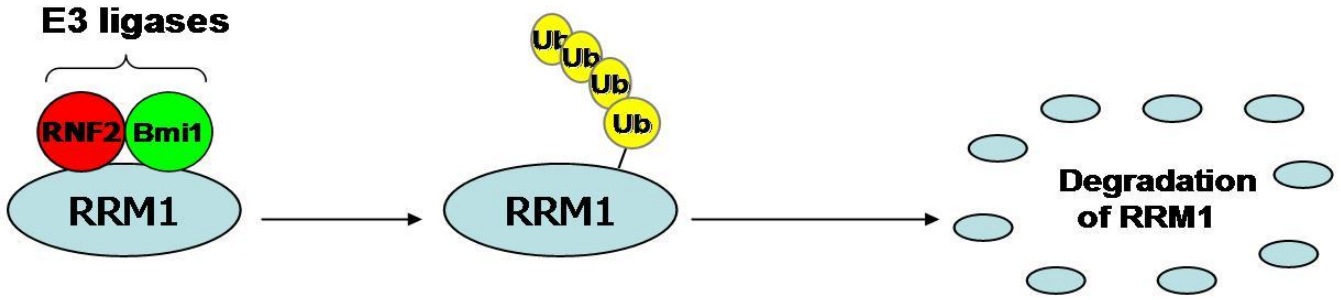
personalized therapy based on ERCC1 and RRM1 expression

Patients with low RRM1/low ERCC1 expression received gemcitabine/carboplatin, patients with low RRM1/high ERCC1 expression received gemcitabine/docetaxel, patients with high RRM1/low ERCC1 expression received docetaxel/carboplatin, and patients with high RRM1/high ERCC1 expression received vinorelbine/docetaxel.

RRM1 (Ribonucleotide Reductase M1)

- Mechanisms that control RRM1 abundance are largely unknown, but may provide an opportunity for optimization of gemcitabine efficacy.
- We have identified that the **E3 ubiquitin-protein ligases RNF2** (RING finger protein 2, Ring1B) and **Bmi1** (B cell-specific moloney murine leukemia virus insertion site 1) are associated with RRM1 by using multiple techniques including yeast two-hybrid screening.

CONCLUSIONS



↑RNF2 and Bmi1→↑RRM1 ubiquitination →↓RRM1 protein level →Gemcitabine sensitivity

↓RNF2 and Bmi1→↓RRM1 ubiquitination →↑RRM1 protein level →Gemcitabine resistance

RNF2 and Bmi1 might be attractive therapeutic targets to overcome gemcitabine resistance in malignancies

Heterogeneous molecular mechanisms in multiple primary melanomas

Colombino et al.

Background: We have studied a series of patients with multiple primary melanoma (MPM) for the involvement of the key-regulator genes in **susceptibility** (*CKDN2A*) and **pathogenesis** (*BRAF*, *cKIT*, *CyclinD1*) of such a disease.

Methods:

Genomic DNA from peripheral blood of 65 MPM patients (57 cases with two primary melanomas, 7 with three, and 1 with four) were screened for germline mutations in $p16^{CDKN2A}$ and $p14^{CDKN2A}$ genes by automated DNA sequencing. Family history for melanoma was investigated: 12 (18%) patients presented at least one additional family member affected.

Paired synchronous and/or asynchronous **MPM tissues** (N=103) from same patients (N=47) were analyzed for somatic mutations in *BRAF* gene and FISH-based amplifications in *cKIT* and *CyclynD1* genes.

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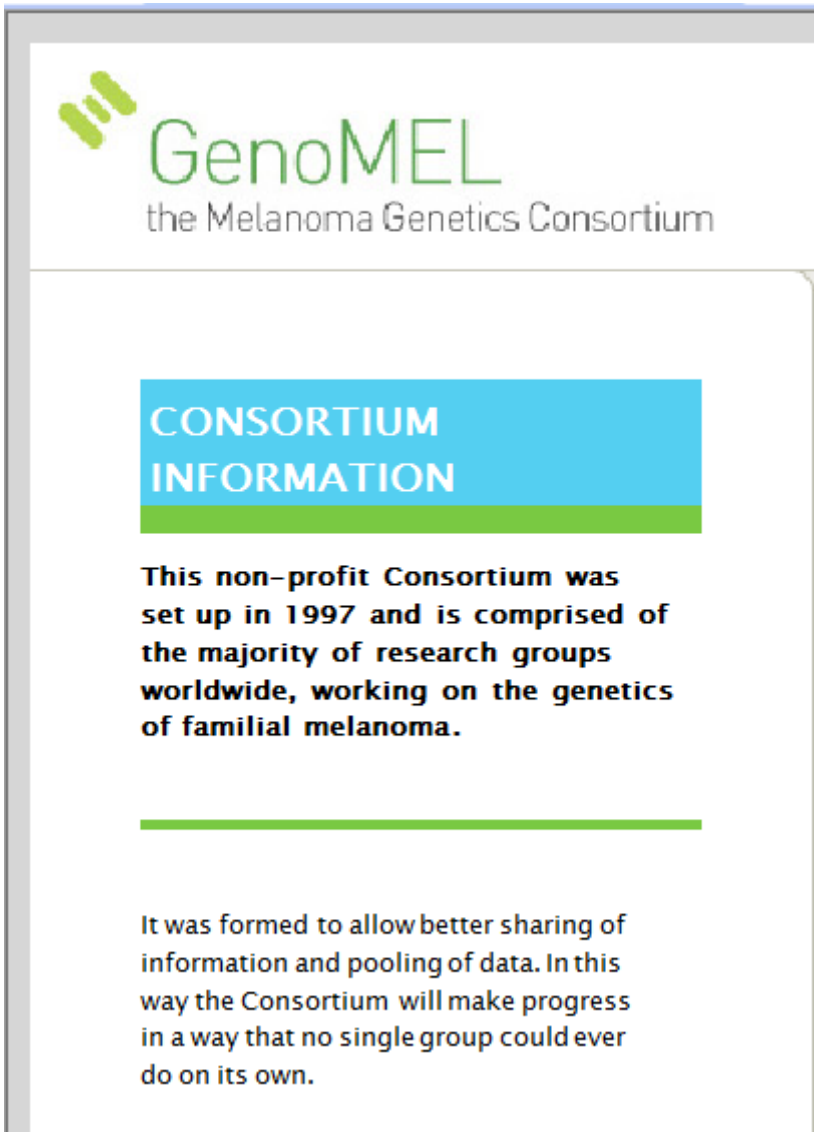
Results: Overall, 7 (11%) different *CDKN2A* germline mutations were identified: 6 in $p16^{CDKN2A}$ and 1 in $p14^{CDKN2A}$. The age of onset was significantly lower and the number of primary melanomas higher in patients with mutations. *CDKN2A* mutations were significantly more frequent in patients with familial history of melanoma (5/12; 42%) compared with patients without (1/53; 4%) ($P<0.01$), and in patients with more than two melanomas (3/8; 37.5%) compared with patients with only two melanomas (4/57; 7%) ($P=0.018$).

Conclusions: Occurrence of at least 3 melanomas (in patients or families), coexistence of MPM and younger age at diagnosis or familial recurrence of melanoma were confirmed to be strong indicators to address patients to *CDKN2A* mutation screening..


GenoMEL
the Melanoma Genetics Consortium
CONSORTIUM INFORMATION
This non-profit Consortium was set up in 1997 and is comprised of the majority of research groups worldwide, working on the genetics of familial melanoma.
It was formed to allow better sharing of information and pooling of data. In this way the Consortium will make progress in a way that no single group could ever do on its own.

Melanoma genetics

- To date there are probably at least 4 genes which underlie predisposition to
- The commonest is a gene on chromosome 9, called CDKN2A, which codes for a cell cycle control protein called p16.

- The second is the CDK4 gene, which codes for the protein to which p16 binds.

- The third is p14ARF. Deletions of this gene have been shown to underlie susceptibility to melanoma and neural tumours. These deletions appear to be very rare.

- Risk of other cancers, clinical features for intiating genetic testing: key for correct patient managment

Heterogeneous molecular mechanisms in multiple primary melanomas

Colombino et al.

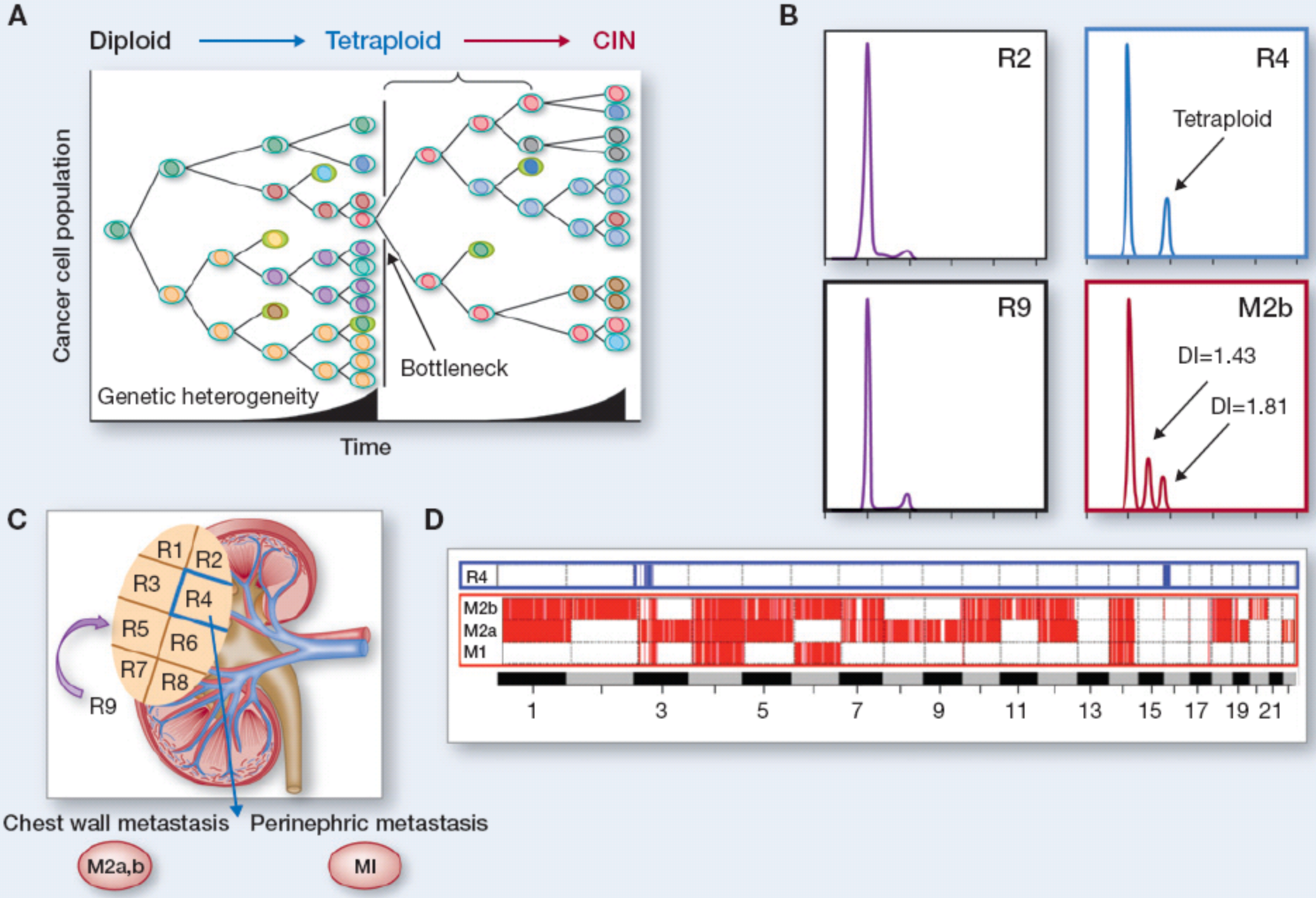
65 patients (57 with two primary melanomas, 7 with three, and 1 with four)

Molecular alterations in 47 patients with available tissues:

- ***BRAF*** **mutations in 38/103 (37%) primary melanomas**
- ***cKIT*** **amplification in 3/98 (3%) primary melanomas**
- ***CyclinD1*** **amplification in 16/98 (16%) primary melanomas**

Poorly consistent distribution of somatic alterations in multiple primary melanomas from same patients

Overall, about half of patients (23/47; 49%) presented molecular discrepancies between first and successive melanomas

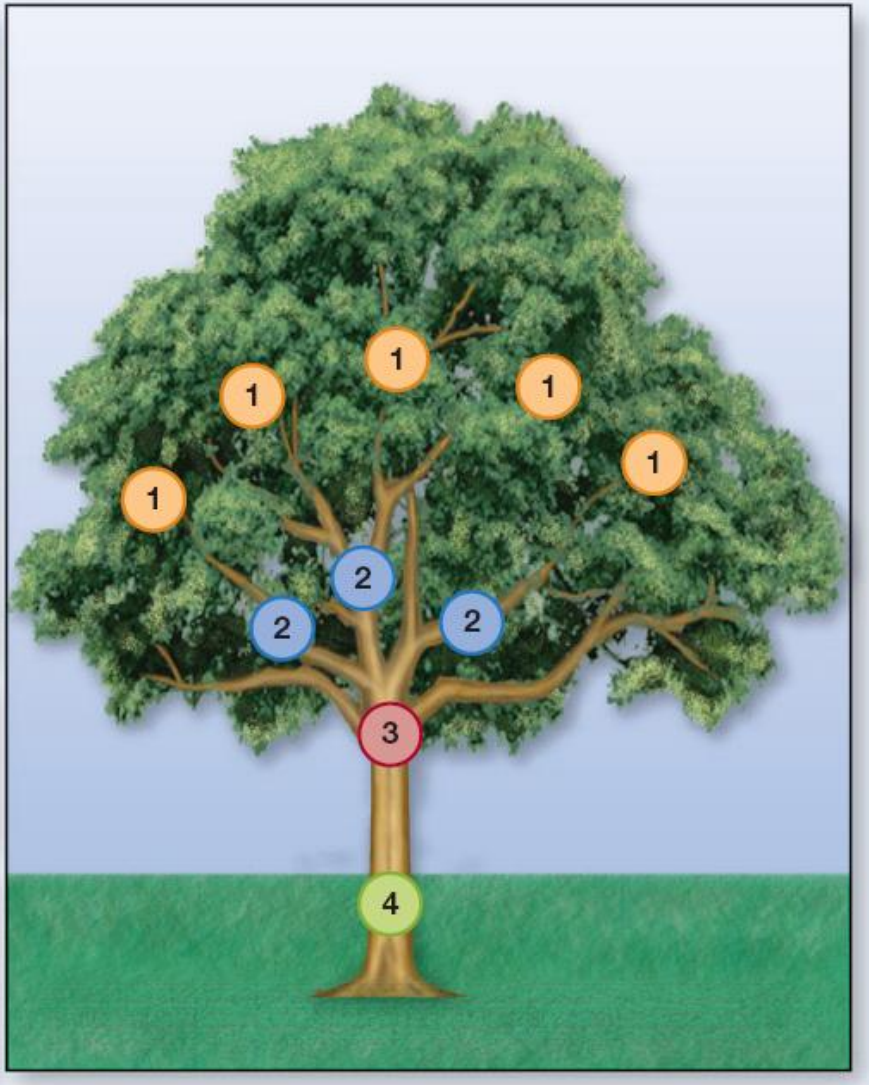


1 **Tracking heterogeneity and bottlenecks:** Development of noninvasive techniques to monitor and track the subclonal dynamics of tumor architecture through treatment may enhance understanding of resistance mechanisms as branches are "pruned" at the expense of outgrowth of other branches harboring heterogeneous resistance mutations (e.g., T790M gatekeeper mutation; ref. 33).

2 **Tumor sampling bias:** Biopsies in 1 region of a heterogeneous primary or metastatic tumor will identify trunk events but may also identify as many or more heterogeneous events not shared by all regions of the tumor or by all tumor subclones. Comparison of paired primary/metastatic samples may enhance the identification of trunk events for therapeutic targeting. Regional genetic ITH may have an impact on *ex vivo* assays of cell phenotypic function.

3 **Drivers of heterogeneity:** Identification of the driver events for genomic instability that may occur at the nexus of the trunk and branch may provide new approaches to limit tumor diversity and adaptation.

4 **Actionable mutations:** Early drivers of disease biology lead to ubiquitous somatic events present in every tumor subclone and tumor region. Such ubiquitous tumor mutations may present more robust therapeutic targets and optimal synthetic lethal targets.



Develop methods to quantify ITH in tumors: Emerging evidence in breast and renal cancer suggests that heterogeneous branched mutations may outnumber common trunk mutations.

Impact of therapy on intratumor heterogeneity: Longitudinal analyses of cytotoxic therapy on ITH will address whether therapy exacerbates branched evolution. Can thresholds of ITH be exploited to improve outcomes?

Heterogeneous molecular mechanisms in multiple primary melanomas

Colombino et al.

65 patients (57 with two primary melanomas, 7 with three, and 1 with four)

***BRAF* mutations in 47 patients with available tissues:**

17 (*36%*) patients with different mutation status

About one third of patients presented a discrepant distribution of *BRAF* mutations in multiple primary melanomas

Need of molecular classification for all lesions in patients with multiple melanoma

The combined expression of CXCR7 and its ligand CXCL12 is marker for unfavorable prognosis in gastric cancer

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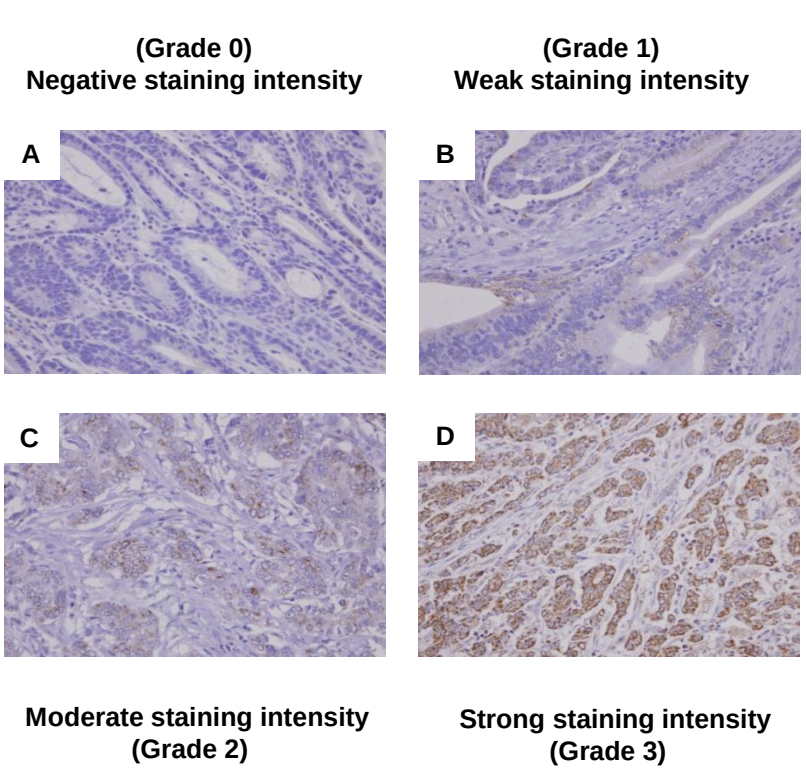
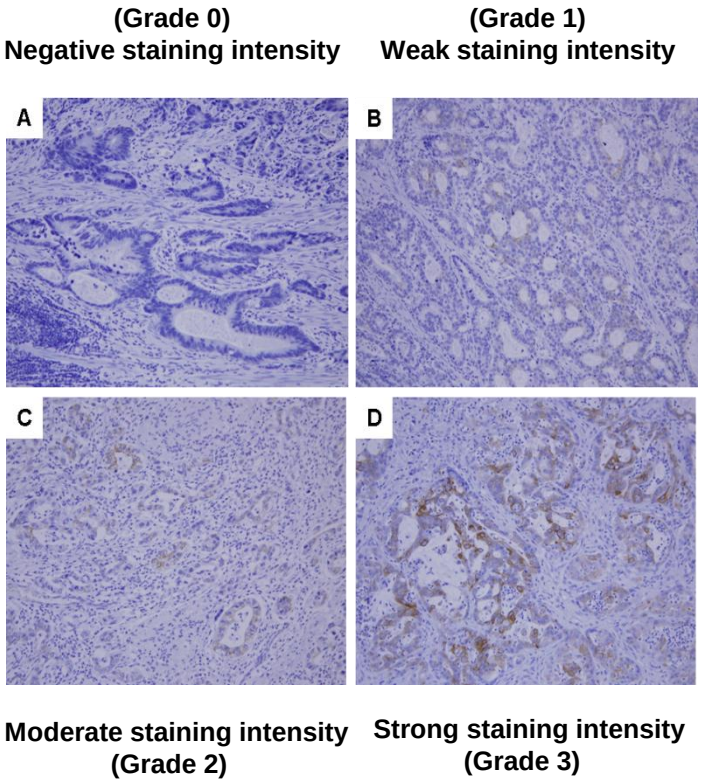
Song Ik Chan,[1] Huang Song Mei,[2] Yun Hwan Jung,[1] Kim Jin-Man,[2] Jo Deog Yeon,[1] Kim Samyong[1]

[1]Department of Internal Medicine and Cancer Research Institute, Chungnam National University School of Medicine; [2]Department of Pathology, Cancer Research Institute and Infection Signaling Network Research Center, Chungnam National University School of Medicine, Daejeon, Korea

CXCR7 and its ligand CXCL12 are differentially expressed in gastric cancer



CXCR7

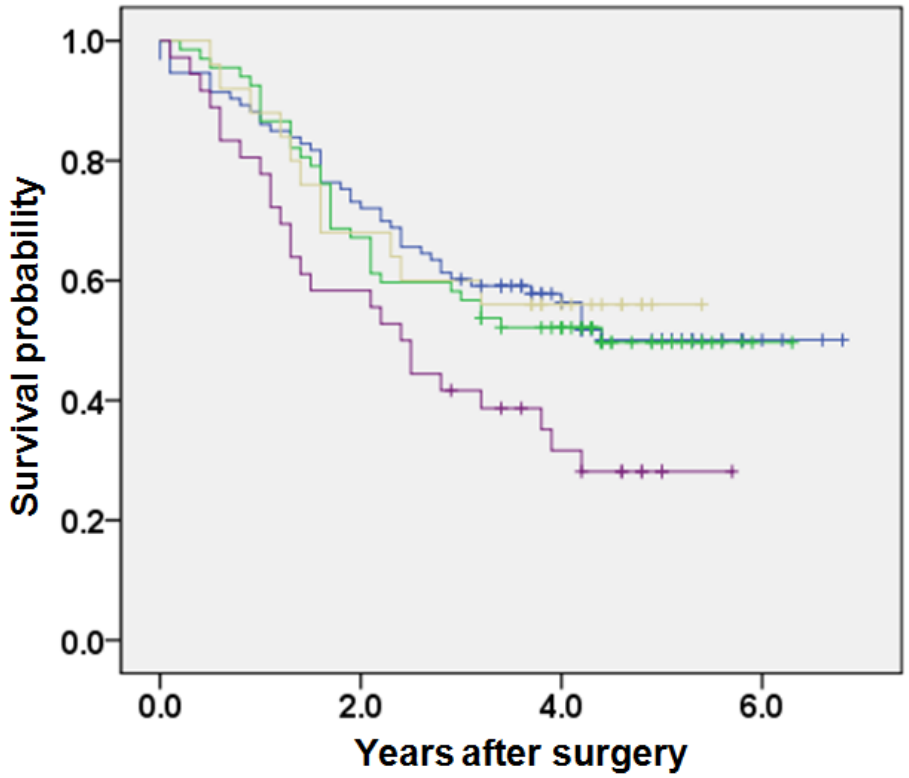


CXCL12

Clinicopathological features of the gastric cancer patients according to CXCR7/CXCL12 expression

	Low CXCR7/ Low CXCL12 (n=93)	High CXCR7/ Low CXCL12 (n=67)	Low CXCR7/ High CXCL12 (n=25)	High CXCR7/ High CXCL12 (n=36)	*P*
Age (years)					
≤ 65/> 65	66/27	43/24	15/10	24/12	0.375
Gender					
Male/Female	63/30	50/17	15/10	27/9	0.599
Depth of invasion					
T1/2/3/4	71/8/14/0	41/12/14/0	19/1/5/0	10/4/21/1	< 0.001
Nodal involvement					
No/Yes	64/29	41/26	15/10	13/23	0.002
Stage					
I,II/III,IV	80/13	55/12	23/2	19/17	0.002
Histology					
Diff/Undiff	46/47	34/33	12/13	13/23	0.298
Tumor location					
Upper	3	5	2	2	0.942
Middle	53	33	13	18	
Lower	36	28	10	16	
Whole	1	1	0	0	
Lymphatic invasion					
No/Yes	70/23	51/16	18/7	31/5	0.373
Venous invasion					
No/Yes	68/25	51/16	20/5	30/6	0.207
Tumor size (cm)					
≤5/>5	81/12	53/14	20/5	23/13	0.006

Patient with high CXCR7/high CXCL12 tumors showed the least favorable prognosis



Low CXCR7/Low CXCL12
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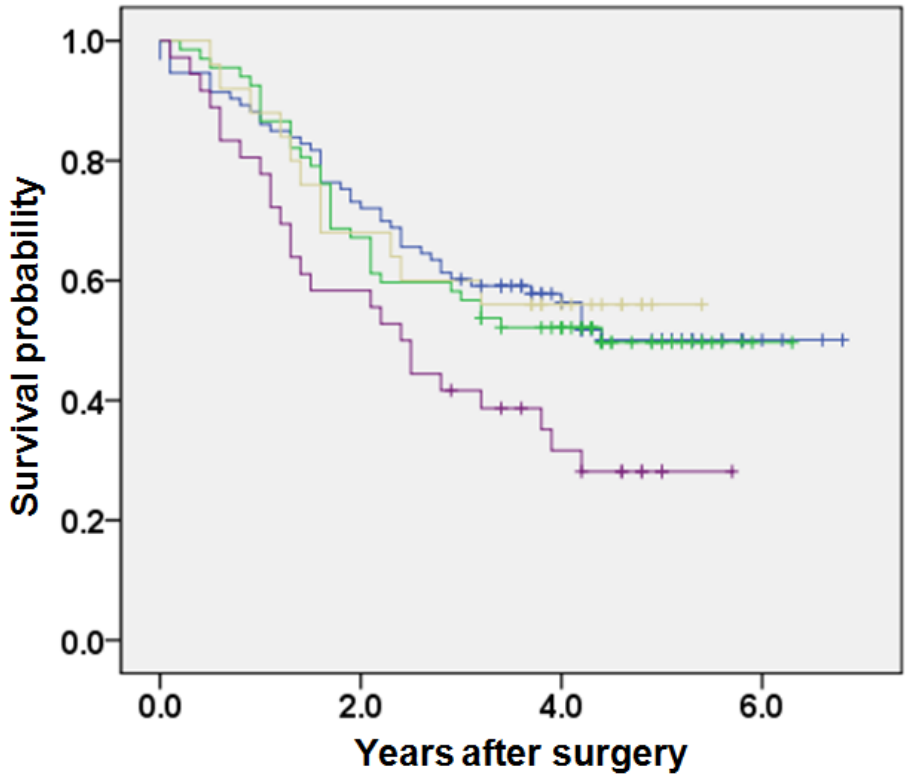
- 5-year survival rate
  30.6% (median, 2.3 years)
  vs. 52.4% (median, not reached; *P*=0.008)

Summary

- **CXCR7 and its ligand CXCL12 are differentially expressed in gastric cancer**
- **CXCR7 and CXCL12 might be useful prognostic factors in gastric cancer, and the combination of high CXCR7 protein expression with high CXCL12 expression suggests a dismal prognosis**
- **Further study to elucidate the role of CXCR7/CXCL12 axis in gastric cancer progression is needed**

Patient with high CXCR7/high CXCL12 tumors showed the least favorable prognosis



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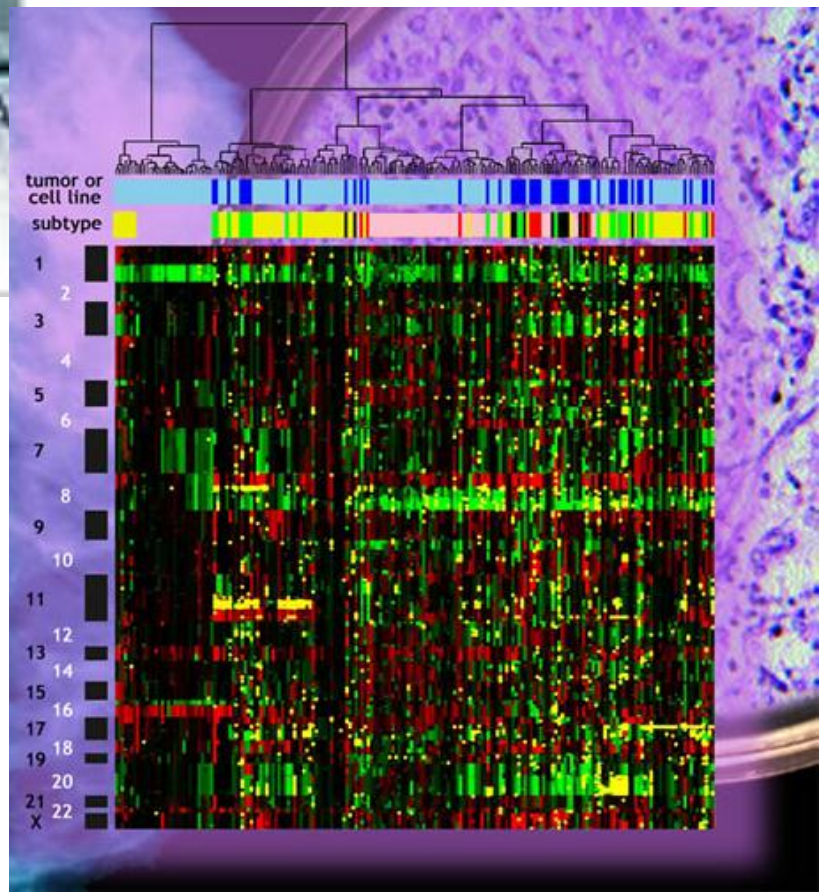
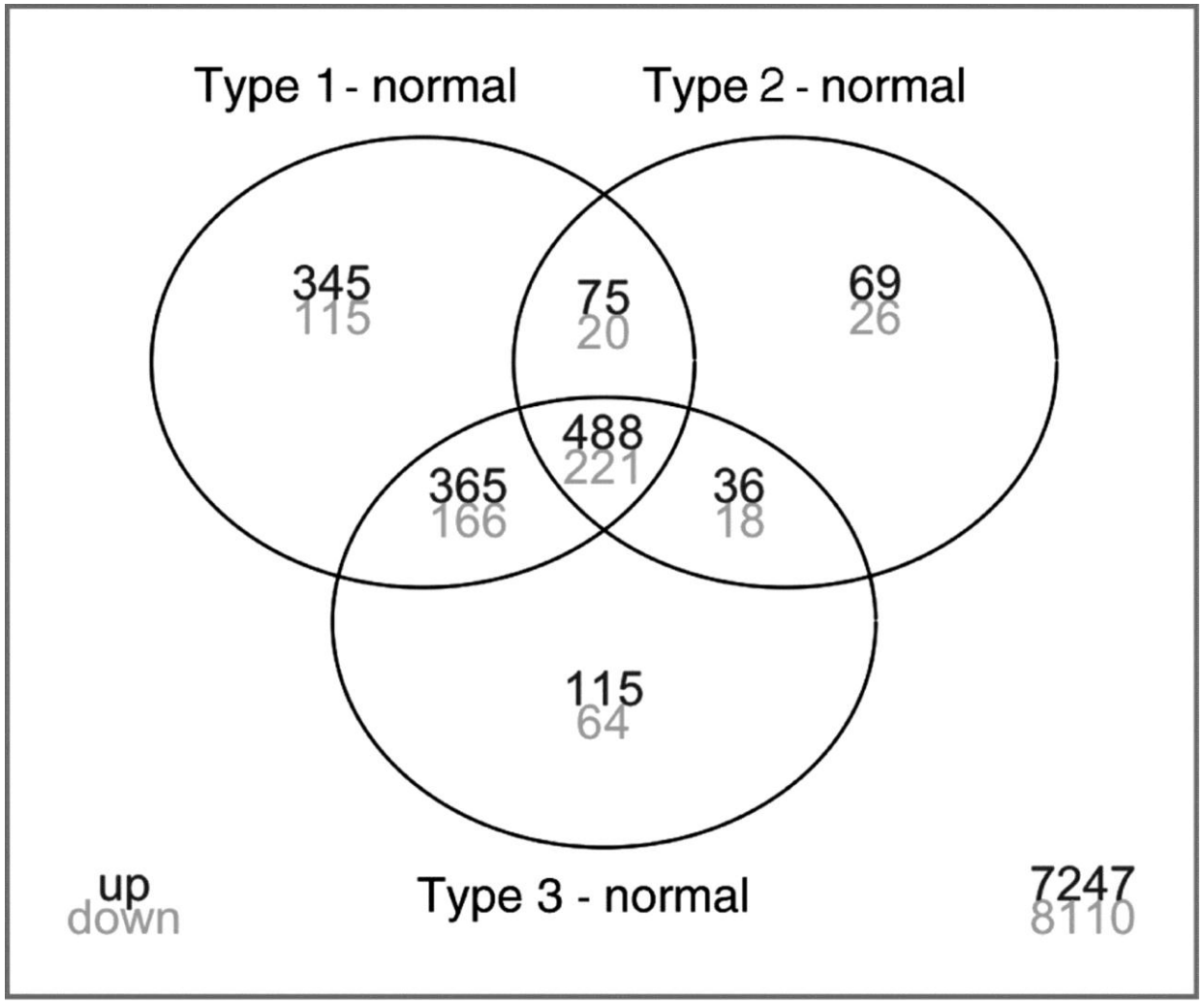
Decoding
Cancer Genomes
Dna, rna, protein,
mirna, methylation,…



2012
Many Novel insights.

Venn diagram showing the distribution of genes that are significantly different between gastric cancer subtypes and normal stomach.



Shah M A et al. Clin Cancer Res 2011;17:2693-2701

Thank you

I sincerely congratulate the Authors