1° Workshop: Diagnostica molecolare e farmaci innovativi

I “driver” molecolari per i nuovi farmaci a bersaglio

Tumori genito-urinari

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SC Oncologia
ASUITS

Trieste, 18 gennaio 2018

CONSIDERAZIONI INIZIALI

- negli ultimi 5-10 aa è cambiato lo scenario per tumori genito-urinari
- nuovi farmaci hanno permesso di modificare la storia di malattia di molti paz non selezionati

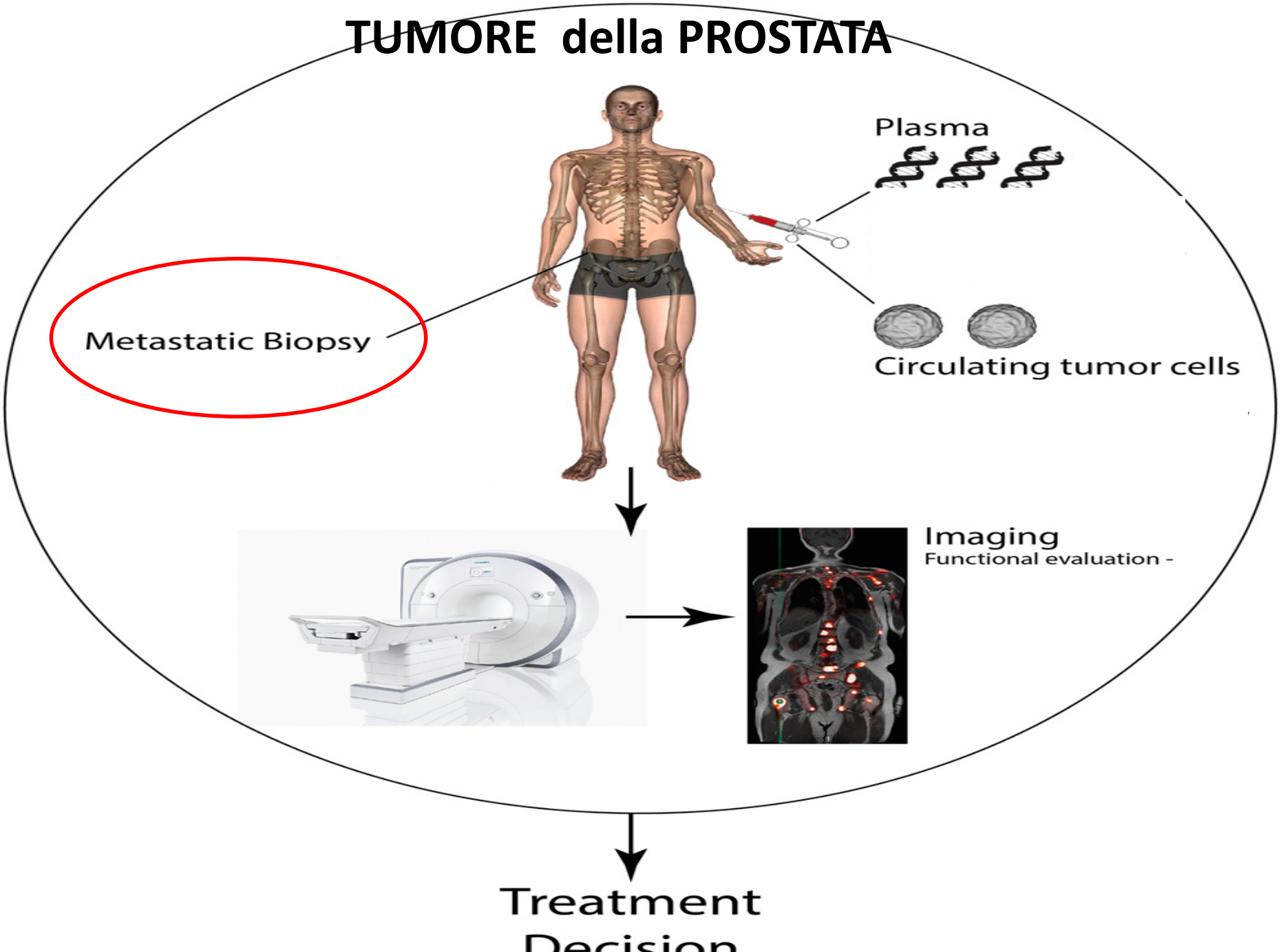
ma

- rimane la necessità di identificare nuovi biomarcatori

Molecular biomarkers

- **Stratificare il rischio**
- **Personalizzare il trattamento**
- **Counseling per paziente e parenti**

TUMORE della PROSTATA



Biopsie tumorali

caratterizzazione molecolare estesa sia nei tumori localizzati che metastatici permette di individuare possibili target o fattori prognostici/predittivi

ERG gene fusion (40%–50%)

AR gene point mutation or amplification (50%–60%)

TP53 mutation or deletion (40%–50%)

PTEN deletion (40%–50%)

RB1 deletion (20%)

alterations in DNA repair genes : BRCA, ATM, MSI …(20%)

H Beltram et al JCO 2016

PARP inhibitori e CRPC

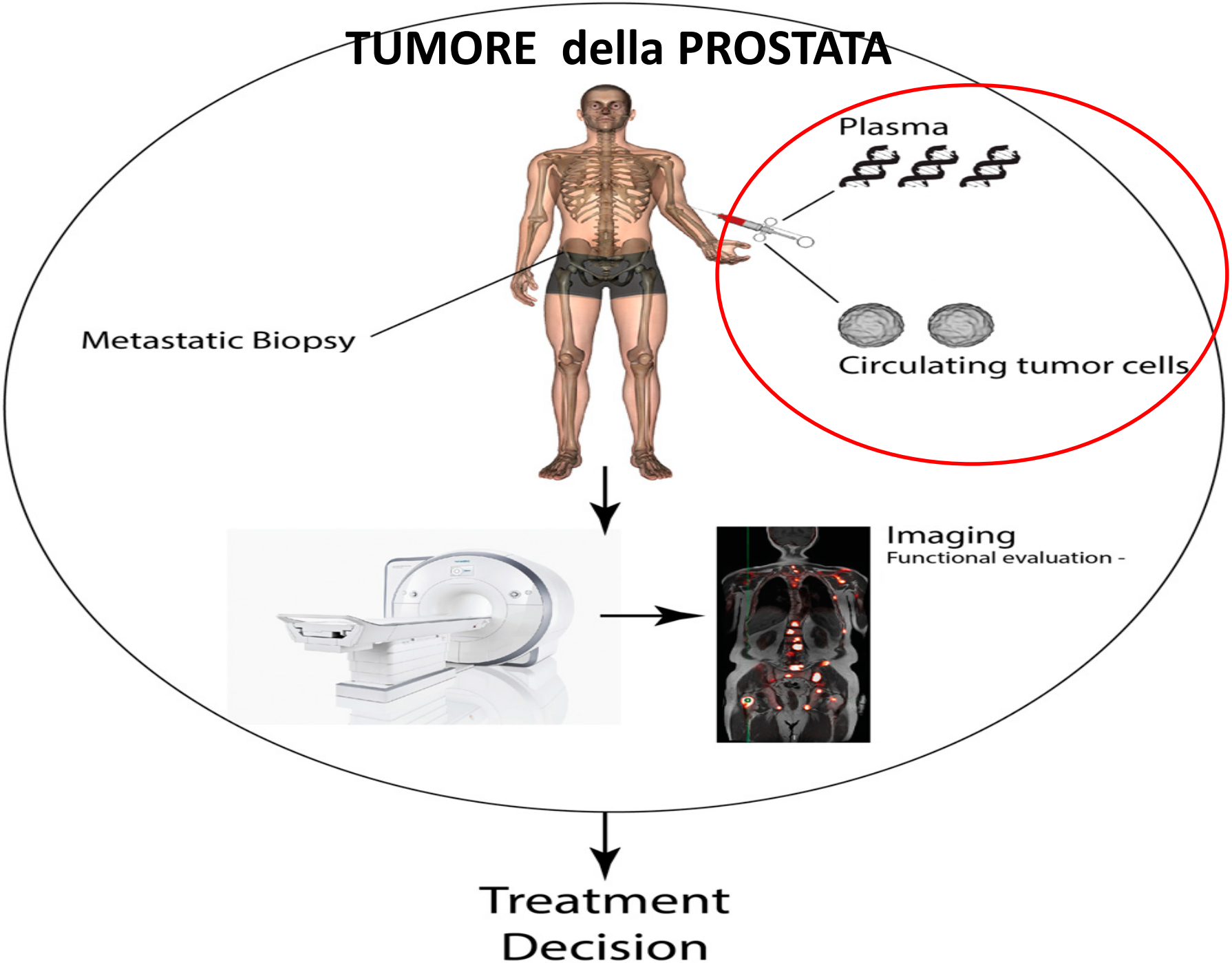
Fase II adattativa di trattamento con olaparib su 49 pz CRPC

con studio prospettico di biomarkers su tessuto e sangue

Overall RR	33%
difetto di meccanismi di riparazione DNA	16/49
RR (DNA repair def)	88%
OS (DNA repair def)	13.8 mo vs 7.5 mo

Mateo J NEJM 2015

TUMORE della PROSTATA



CTC o DNA circolante: quale aiuto nella pratica clinica

- ✓ **CTC circolanti:**

  studio COU-301 : CTC e LDH markers surrogati di OS [1]

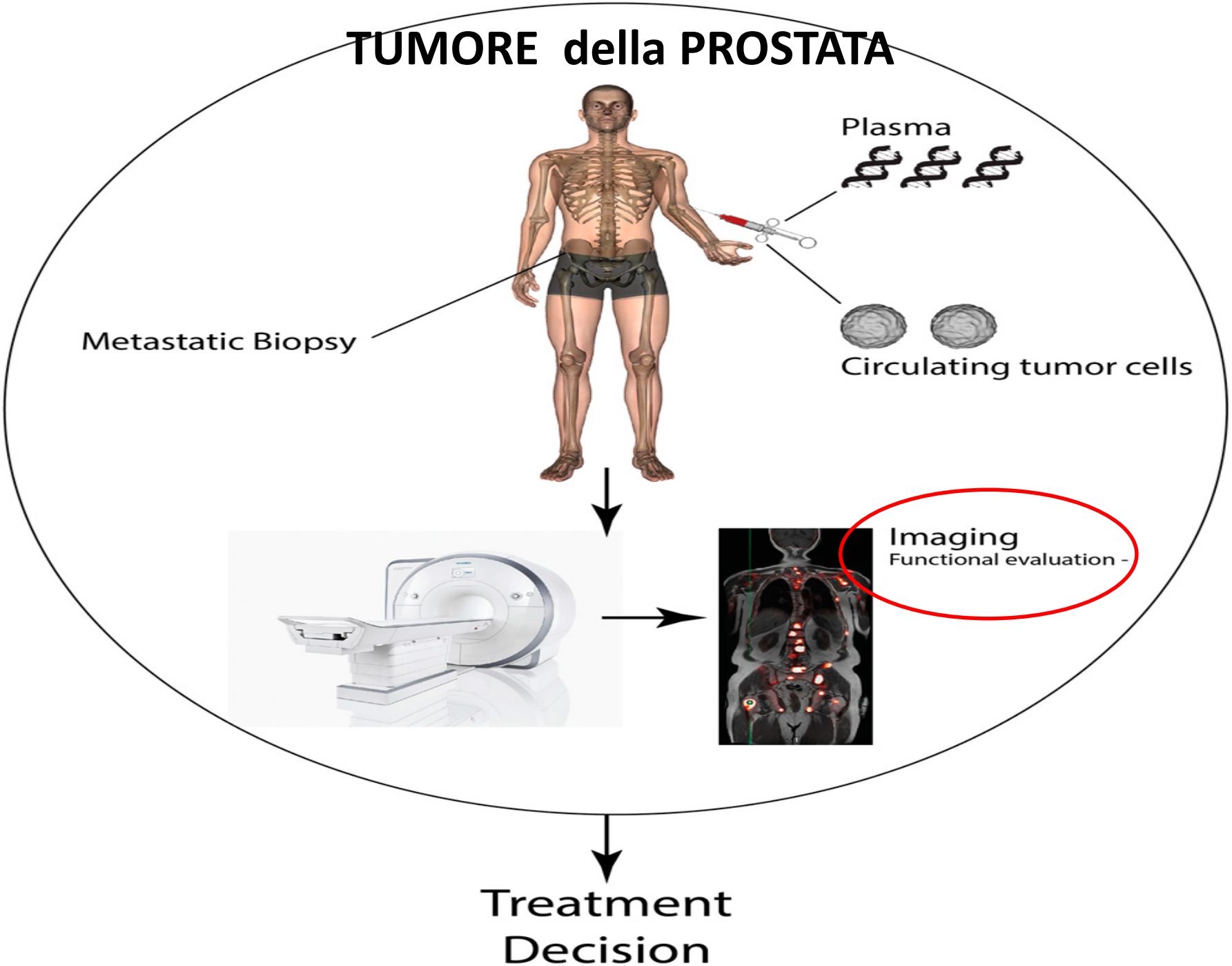
- ✓ **DNA tumorale circolante:**

  comparsa di mutazioni di AR (T878A o L702H) in 15-20% di pz anticipano di mesi PD radiologica [2]

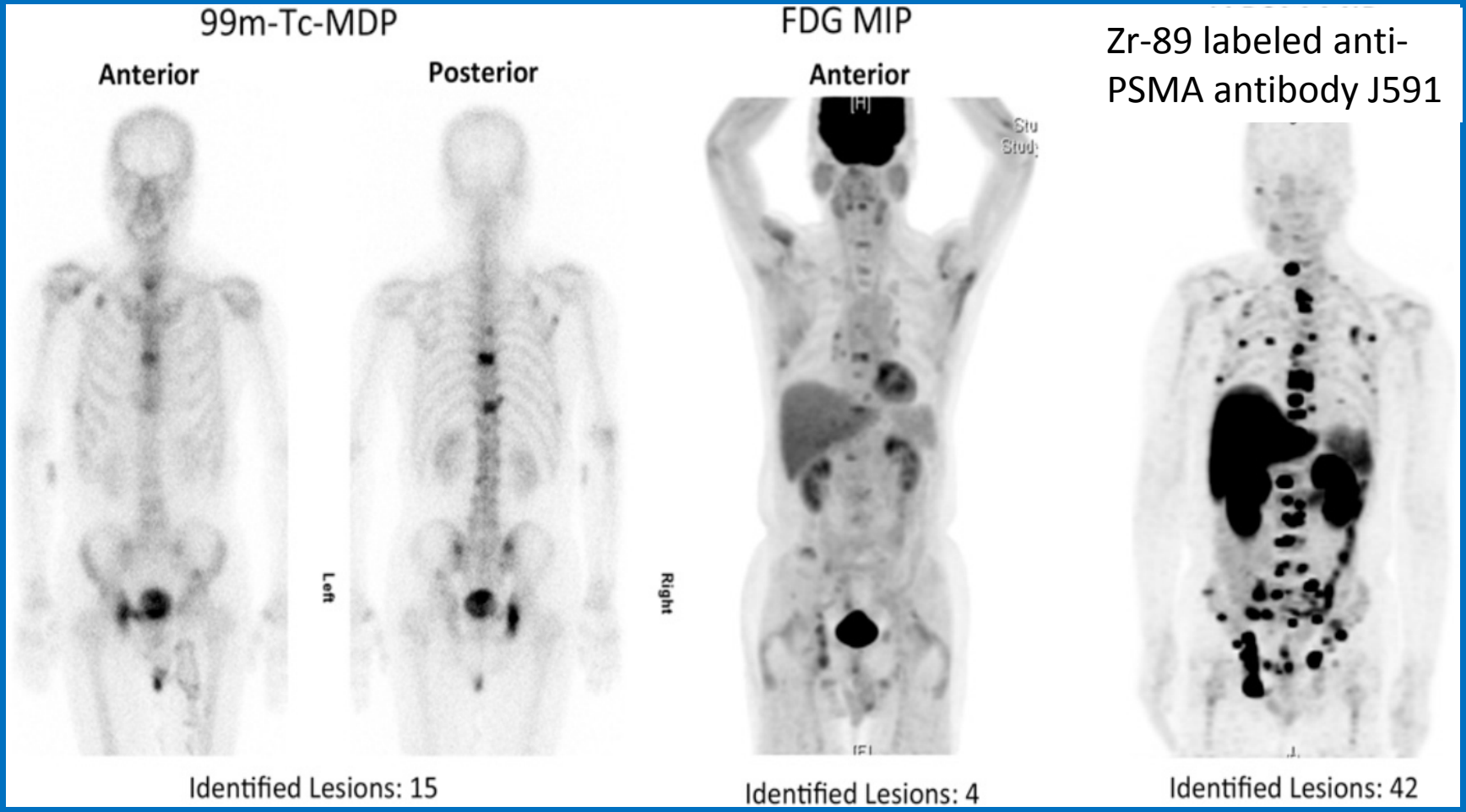
- ✓ **Mutazione AR-V7 in CTC → marcatore di R a nuovi antiandrogeni ma non ai taxani (PRIMCAB trial, sviluppo di nuovi farmaci diretti contro AR-V7)** [3]

[1] Scher JM JCO 2015
[2] Romanel A Sci Transl Med 2015
[3] Antonarakis ES JAMA Oncolo2015

TUMORE della PROSTATA



Molecular imaging



H Beltram et al ASCO 2016

Molecular imaging

potrebbe migliorare:

- **la comprensione del comportamento biologico del tumore**
- **stadiazione iniziale per evitare chirurgie inutili sul primitivo**
- **evidenziare precocemente recidive locali**
- **predirre e monitorare la risposta al trattamento**
- **affiancare lo sviluppo di nuove strategie terapeutiche**

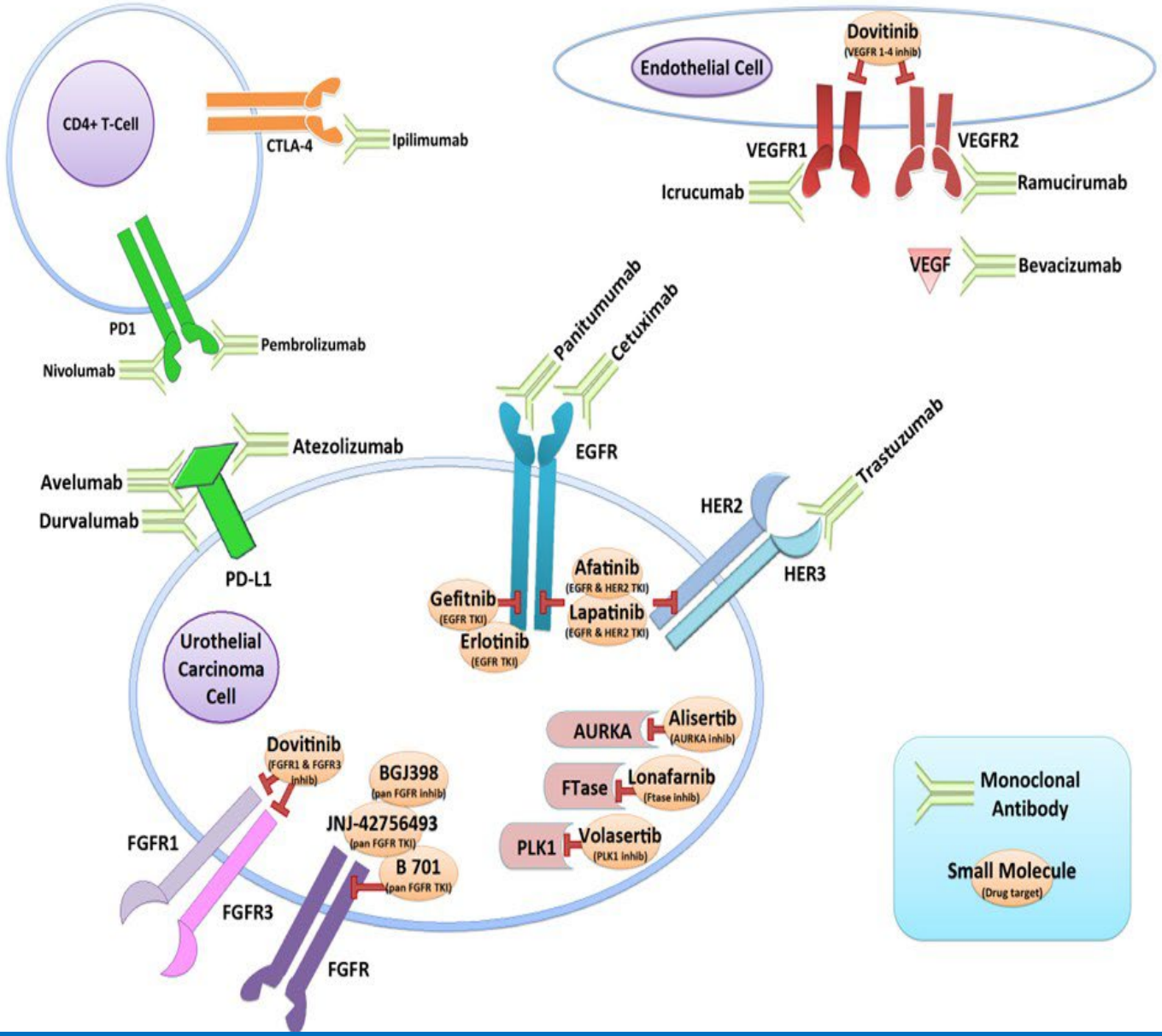
Molecular biomarkers

E nei tumori uroteliali e renali ?

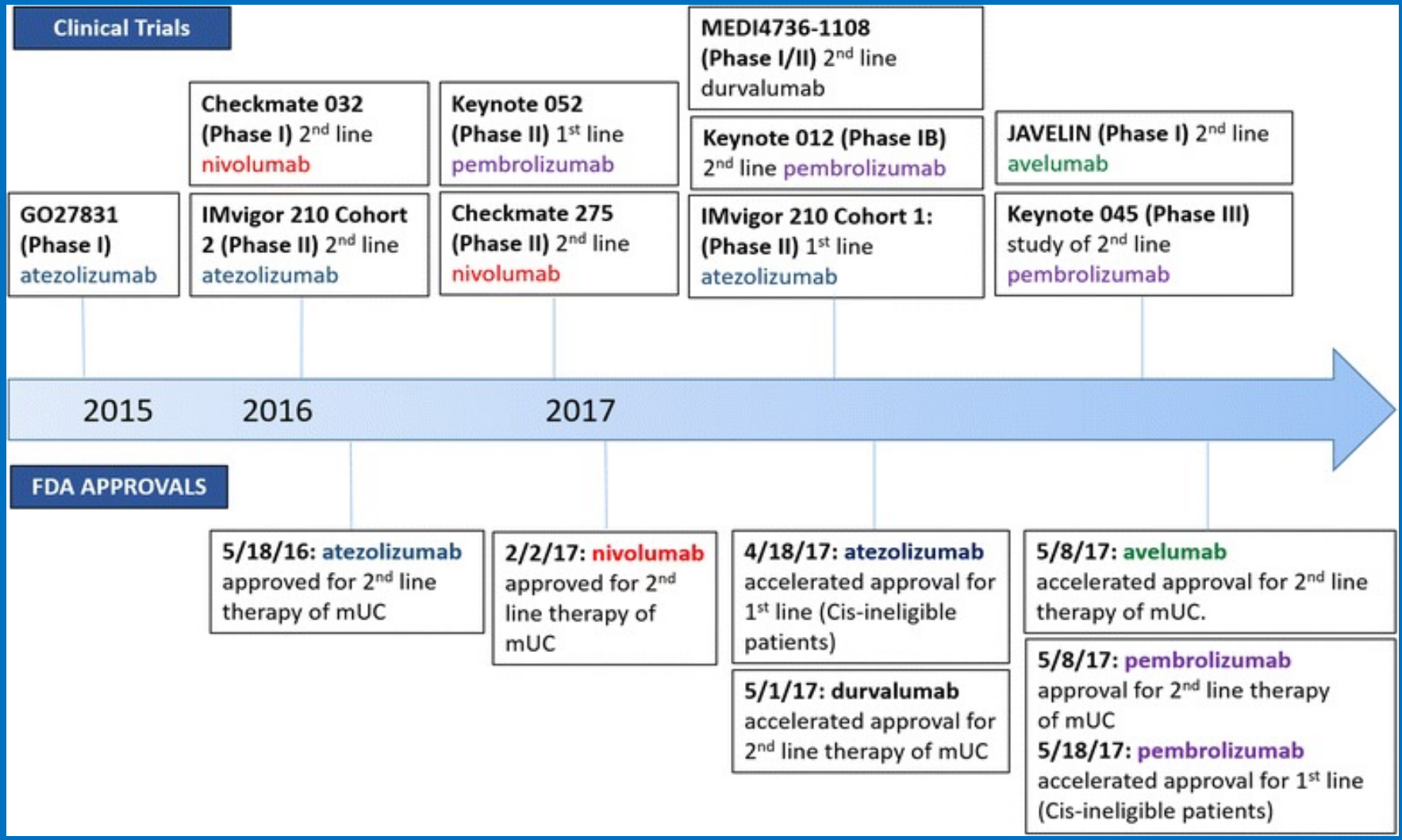
Tumore uroteliale

TCGA subtypes	Molecular markers	Prognosis	Chemo-response	Anti-PD-L1 Response %	Chemorestistance related genes
Cluster I (papillary like)	PPAR Y activity FGFR3 activating Mutations ERB2,FOXA1	Good to intermediate	Sensitive	10	
Cluster II	p53 activity signalimg Low expression of markers of cell cycle and proliferation		Resistant	35	Mutations ERCC2, BRCA1 e 2
Cluster III (basal-Squamous-like)	p53, activity, TKR5, EGFR	Poor	Sensitive	16	
Cluster IV (claudine low)	Emt markers, EGFR amplif, RB1 mut		Non assessed	20	

Neoplasie uroteliali

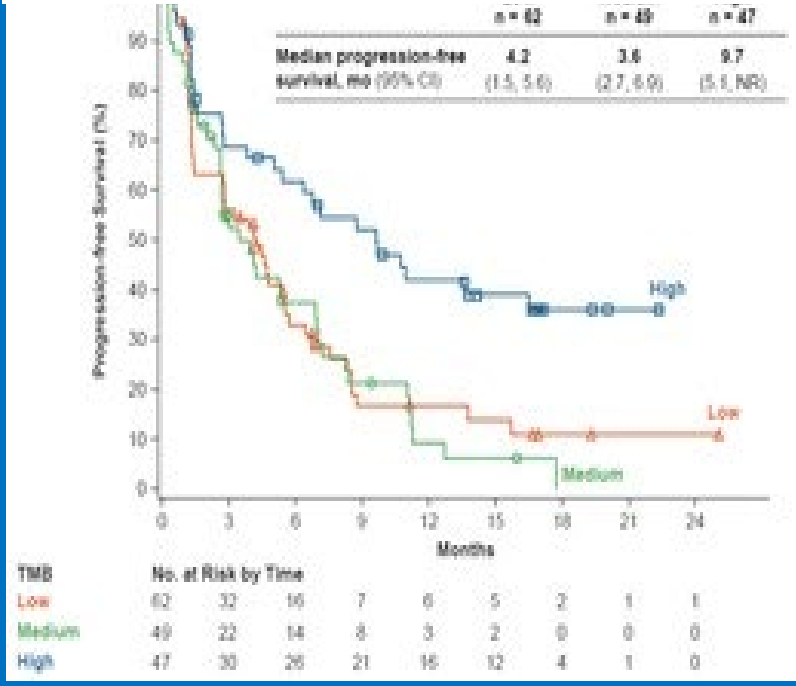


IMMUNOTERAPIA:
LA RIVOLUZIONE DEL PARADIGMA

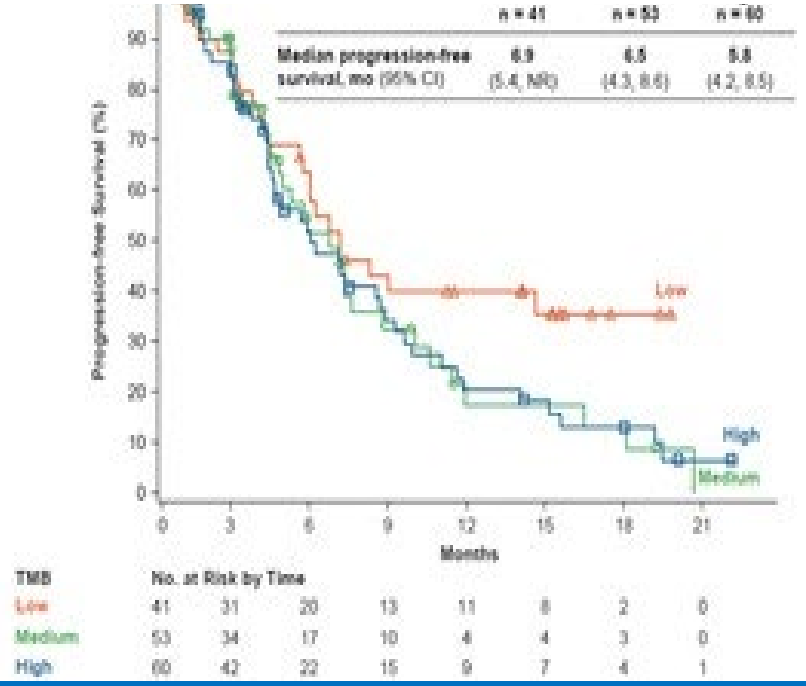


TUMOR MUTATION BURDEN AND PFS

nivolumab

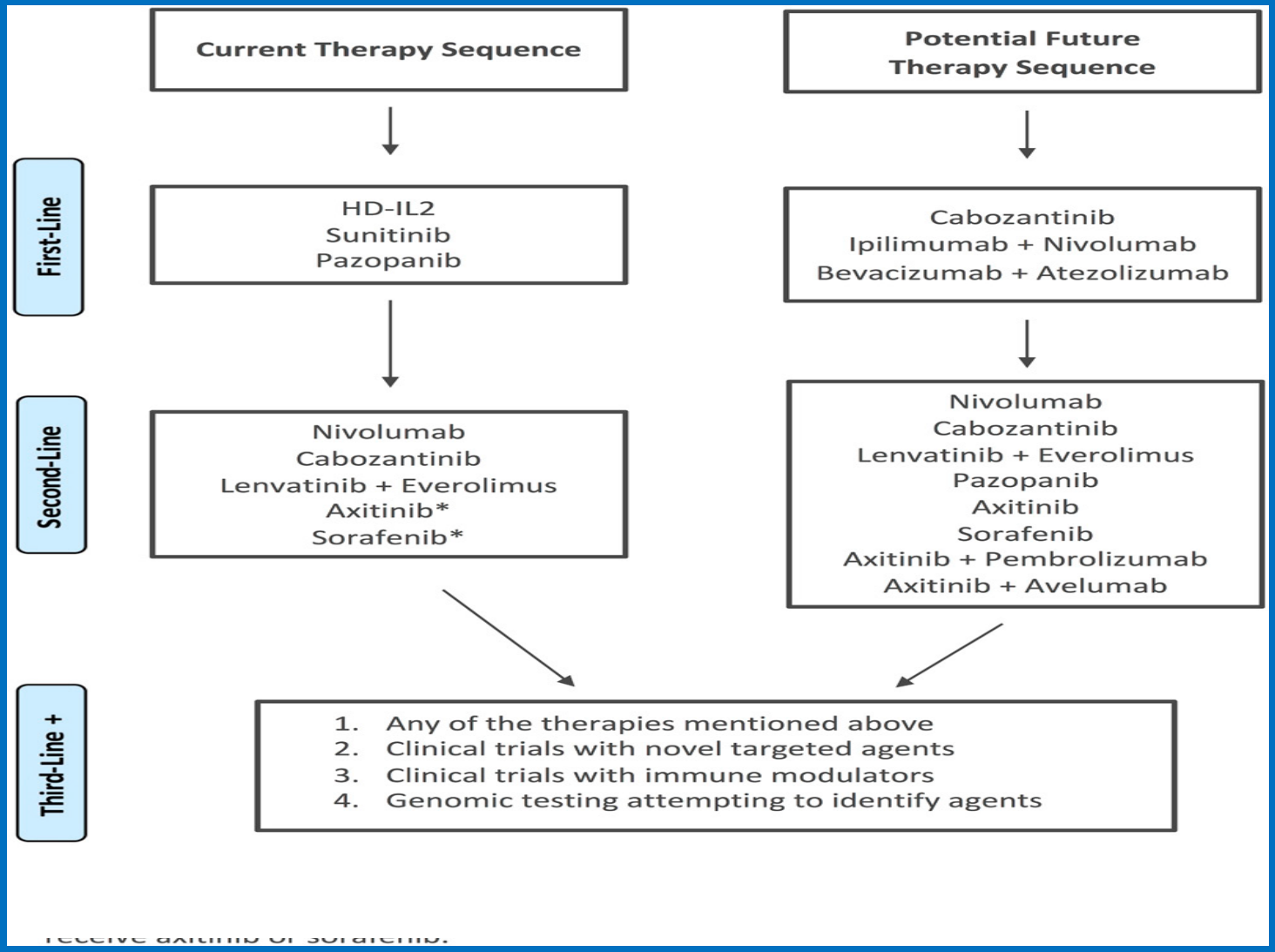


chemotherapy



Carbone, D. et al. *NEJM*. 2017

Ca renale: scenari futuri

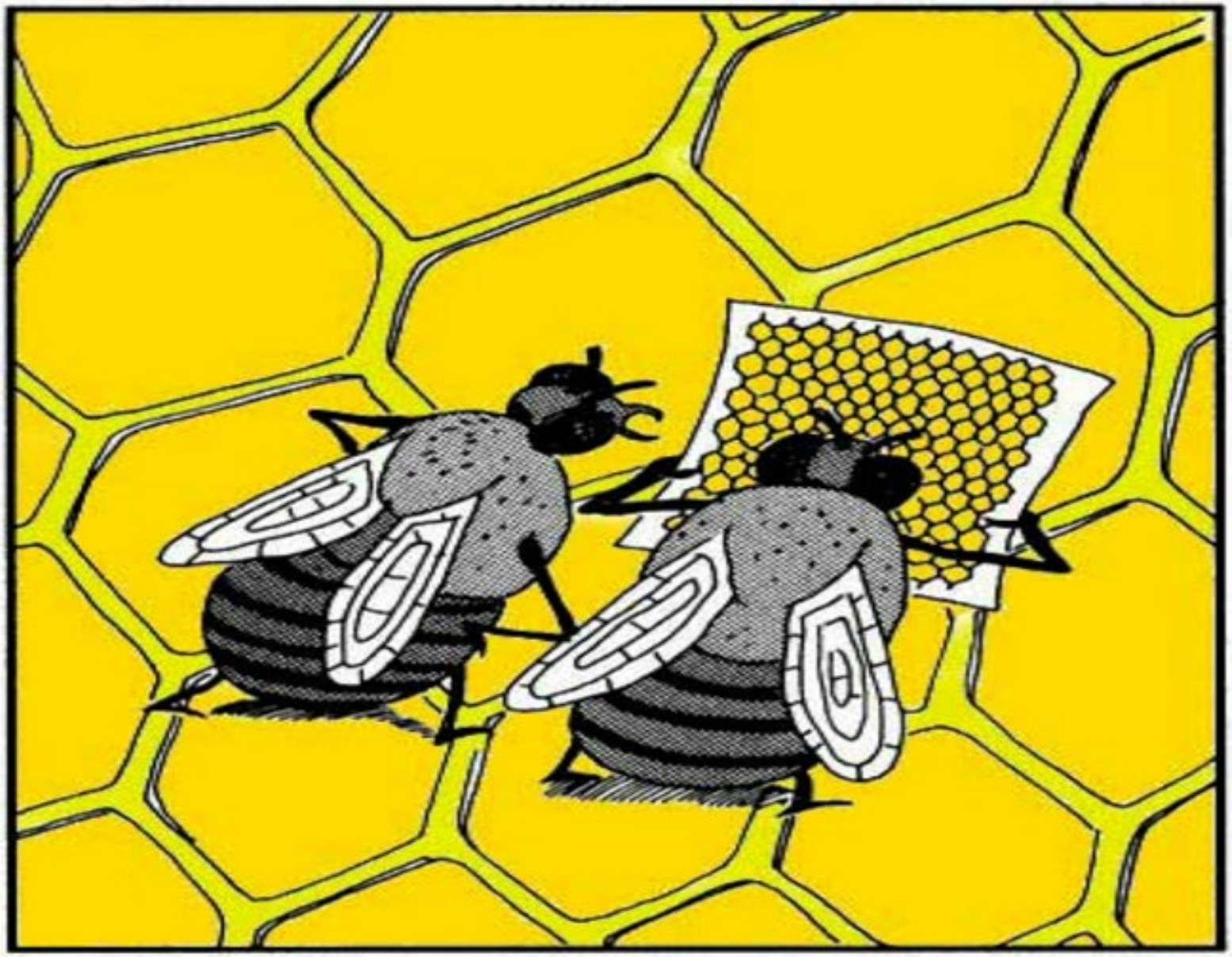


Gill DM, ASCO 2017

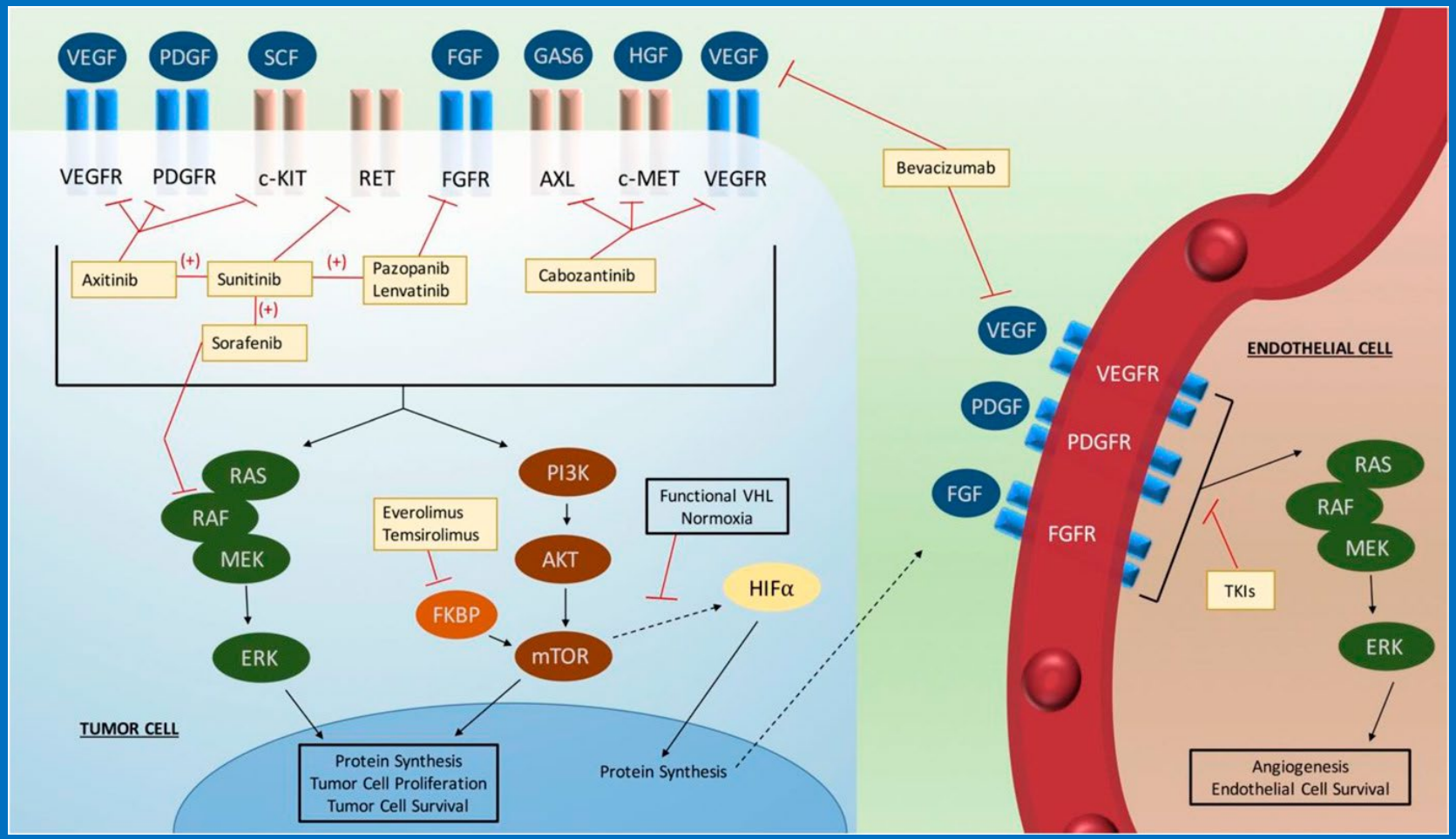
CONCLUSIONI

- ✓ La caratterizzazione molecolare estesa ″entra″ nella pratica clinica
- ✓ Necessità di modificare metodologia della ricerca
- ✓ Possibili suggerimenti per sequenze ed associazioni
- ✓ Immunoterapia: necessità di fattori predittivi per migliore selezione dei paz

„State of the art per Nadia Harbeck“



Neoplasie renali

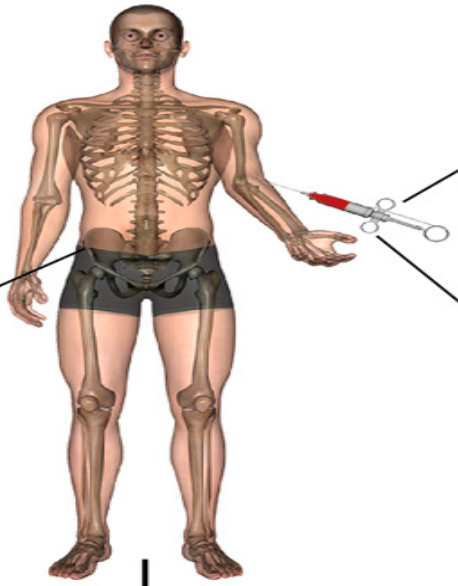


CONCLUSIONI

- La caratterizzazione molecolare estesa elemento da integrare nella pratica clinica
- ha portato Importanza di sviluppare fattori predittivi di risposta
- Necessità di collaborazione dai grandi studi randomizzati ai piccoli studi su mutazioni rare
- Nuove tecniche di caratterizzione molecolare(su cellule e tessuto) possono dare ulteriore sviluppo di nuove terapie e maggiore personalizzazione

CONCLUSIONI

- ✓ Nuove tecniche di caratterizzazione molecolare (su cellule e tessuto) possono dare ulteriore impulso allo sviluppo di nuove terapie e maggiore personalizzazione
- ✓ Mandatorio ricercare fattori predittivi di risposta per modulare la scelta terapeutica
- ✓ Immunoterapia: al momento non abbiamo criteri «clinici» o biomarcatori per la selezione dei paz



Plasma

Targeted analysis of
circulating tumor DNA
e.g. AR (Romanel et al, 2015),
BRCA1/2, ATM

Circulating tumor cells

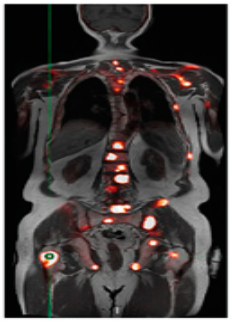
Targeted analysis of CTCs
e.g. AR-V7(Antonarakis et al, 2014),
expression profiles, single cell seq.

Metastatic Biopsy

Whole exome, transcriptome
(Robinson et al, 2015)

Targeted analysis of actionable
genomic lesions (Mateo et al, 2015)

DNA Methylation (Beltran et al, 2016)



Imaging

Functional evaluation -
e.g. NaF, DHT, PSMA

Treatment Decision

The cancer genome atlas (TCGA)

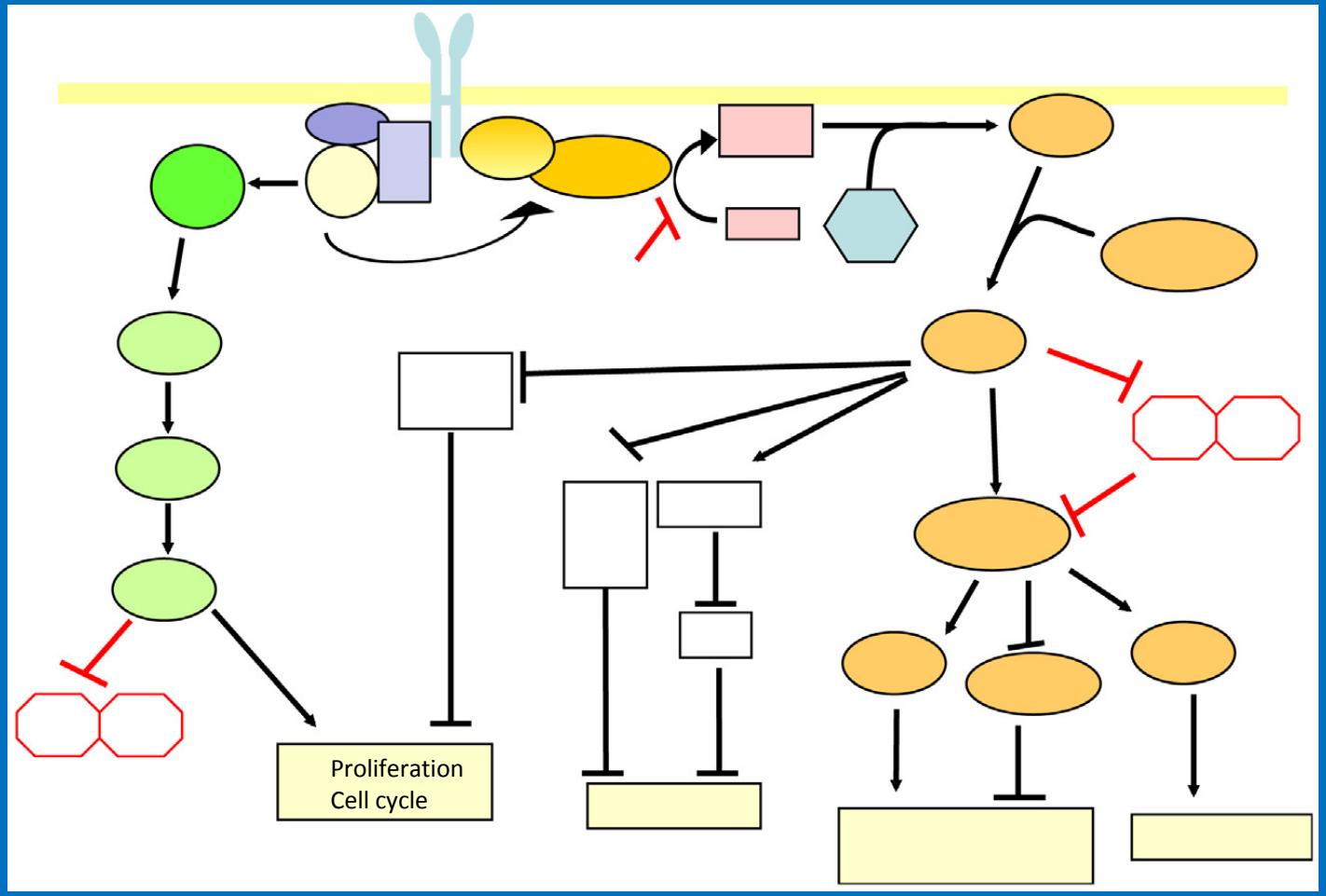
MIBC		
BASAL	Attivazione p63,mutazione EFGR	Differenziazione squamosa, peesentazione + aggressiva, ma prognosi intermedia
LUMINAL	PPAR gamma, ER, FGFR mutato	Possibile risposta a inibitori di FGFR Istotipo a maggiore aggressività
P-53 like		Resistente a NAC

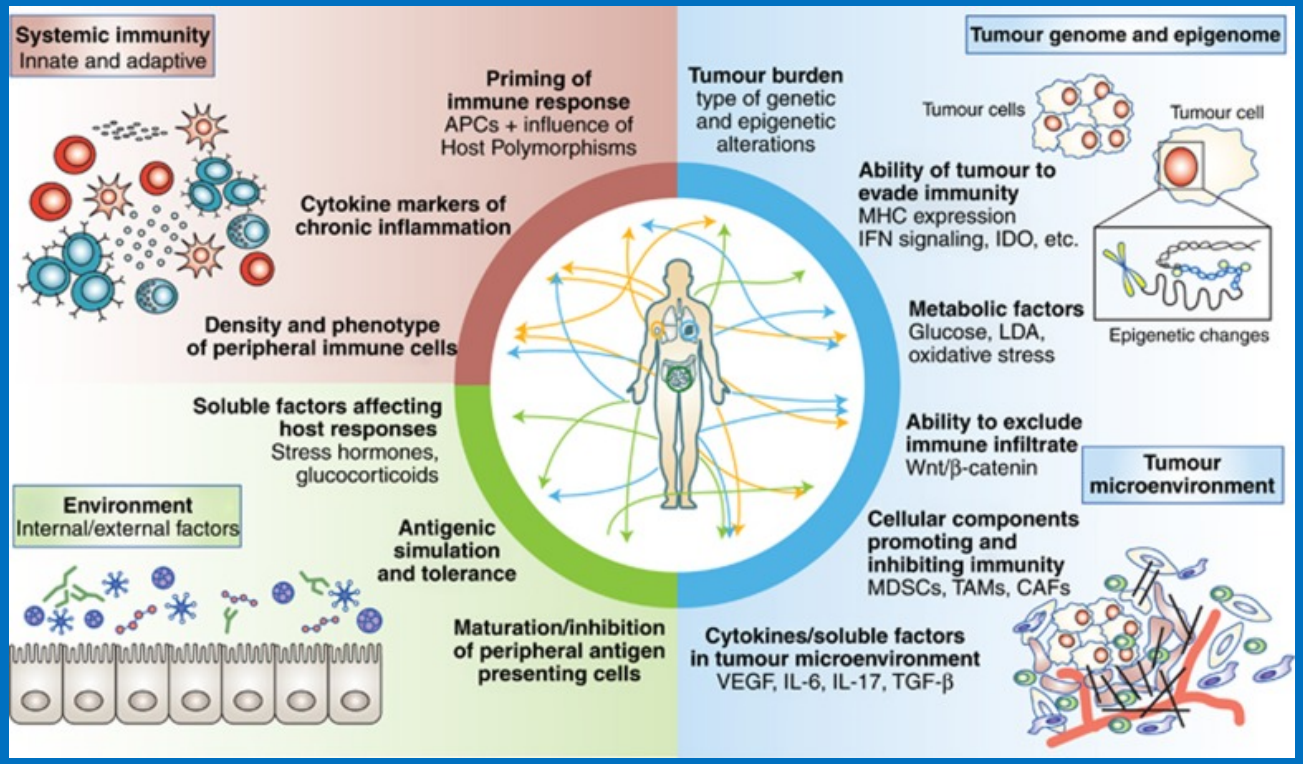
Tumori vescicali hanno un più alto carico di mutazioni rispetto a tumori uroteliali Vie urinarie superiori

	FDA	EMA	AIFA
Atezolizumab (anti PD-L1)	1°st unfit	1°st	
Pembrolizumab (anti PD1)	1° unfit	-	
Nivolumab (anti PD1)			
Avelumab (anti PD-L1)			
Durvalumab (anti PD-L1)			

- **7: Summary of Other Targets of Interest**
- OS = Overall Survival, ORR = Objective response rate, PFS = Progression free survival
- **Study ID Experimental Agent Drug Class Treatment Combination Phase Patient Population AE Outcome Biomarker Selection Targets**
- Volasertib Polo-like
  - Inhibitor
  - (PLK)

- [72] Single agent II Second line G3-4 20-28%
- RR = 14%, PFS =
- 1.4 months, OS
- = 8.5 months
- PLK1
- Lonafarnib
- [73]
- Farnesyl
- transferase
- inhibitor
- Lonafarnib +
- Gemcitabine II Second line G3-4 36%
- ORR=32%,
- TTP=7months,
- OS=11.5months
- Farnesyl
- transferase
- NCT02109328 Alisertib
- [74]
- Aurora
- kinase A
- inhibitor
- Single agent II Second line
- G3-4 41-54%
- with
- 2 treatment
- deaths


Proliferation
Cell cycle


Systemic immunity
Innate and adaptive
Priming of immune response
APCs + influence of Host Polymorphisms
Cytokine markers of chronic inflammation
Density and phenotype of peripheral immune cells
Soluble factors affecting host responses
Stress hormones, glucocorticoids
Environment
Internal/external factors
Antigenic simulation and tolerance
Maturation/inhibition of peripheral antigen presenting cells
Tumour burden
type of genetic and epigenetic alterations
Tumour genome and epigenome
Tumour cells
Tumour cell
Ability of tumour to evade immunity
MHC expression
IFN signaling, IDO, etc.
Epigenetic changes
Metabolic factors
Glucose, LDA, oxidative stress
Ability to exclude immune infiltrate
Wnt/β-catenin
Tumour microenvironment
Cellular components promoting and inhibiting immunity
MDSCs, TAMs, CAFs
Cytokines/soluble factors in tumour microenvironment
VEGF, IL-6, IL-17, TGF-β

	Advantages	Disadvantages
PD-L1 Immunohistochemistry	• Most well-characterized biomarker to date • Rapid turn around time from biopsy • IHC assays are standardized specific to each therapy • Relatively inexpensive	• Discordant results across studies • Poor negative predictive value: responses seen in PD-L1 negative tumors • Multiple antibodies in use to detect PD-L1 • Unclear if composite score or tumor cell score is more reflective of the tumor microenvironment • Biomarker is dynamic over time and does not reflect PD-1/PD-L1 interactions in tumor draining lymph nodes • Does not assess status of the immune microenvironment
TCGA Subtyping	• Evidence of increased immunotherapy response in luminal cluster II subtypes with atezolizumab • Basal cluster I subtype demonstrated increased ORR with nivolumab therapy • Distinct classifications based on tumor gene signatures (i.e. few patients with gene signatures between groups)	• Multiple gene cluster assays used, difficult to standardize • TCGA subtyping in patients treated with immunotherapy is limited to small numbers in each cohort (<60 patients in IMVigor study) • May require deep sequencing to appropriately identify the TCGA subtype • Responses are achieved in all 4 TCGA clusters, suggesting a low negative predictive value • Does not assess status of the immune microenvironment
Tumor Mutational Burden	• Clear examples of durable responses (> 6 months) in patients with high mutation burden • Correlation demonstrated in subgroup analyses between tumor mutation burden and overall response rates with atezolizumab and pembrolizumab	• Difficult to standardize between sequencing assays • Relative weight of SNPs and translocations not yet elucidated • Relationship between tumor mutation burden and neoantigen burden is still undefined • Depth of sequencing required to predict responders vs nonresponders undetermined • Evolution of tumor over time may change the relative mutation burden • Does not assess status of the immune microenvironment
Immune Cell Gene Expression Profiling	• Higher reproducibility relative to PD-L1 IHC to predict immunotherapy responses • Only biomarker assessing immune cell status rather than tumor characteristics • Correlated with response to therapy in subgroup analyses of nivolumab and pembrolizumab trials	• No standardized commercially available gene panel as of yet. Multiple gene panels currently available (T-cell panel, combined T-cell tumor cell panel, IFN-γ specific) • Insufficient negative predictive value: responders seen in all groups • Cost

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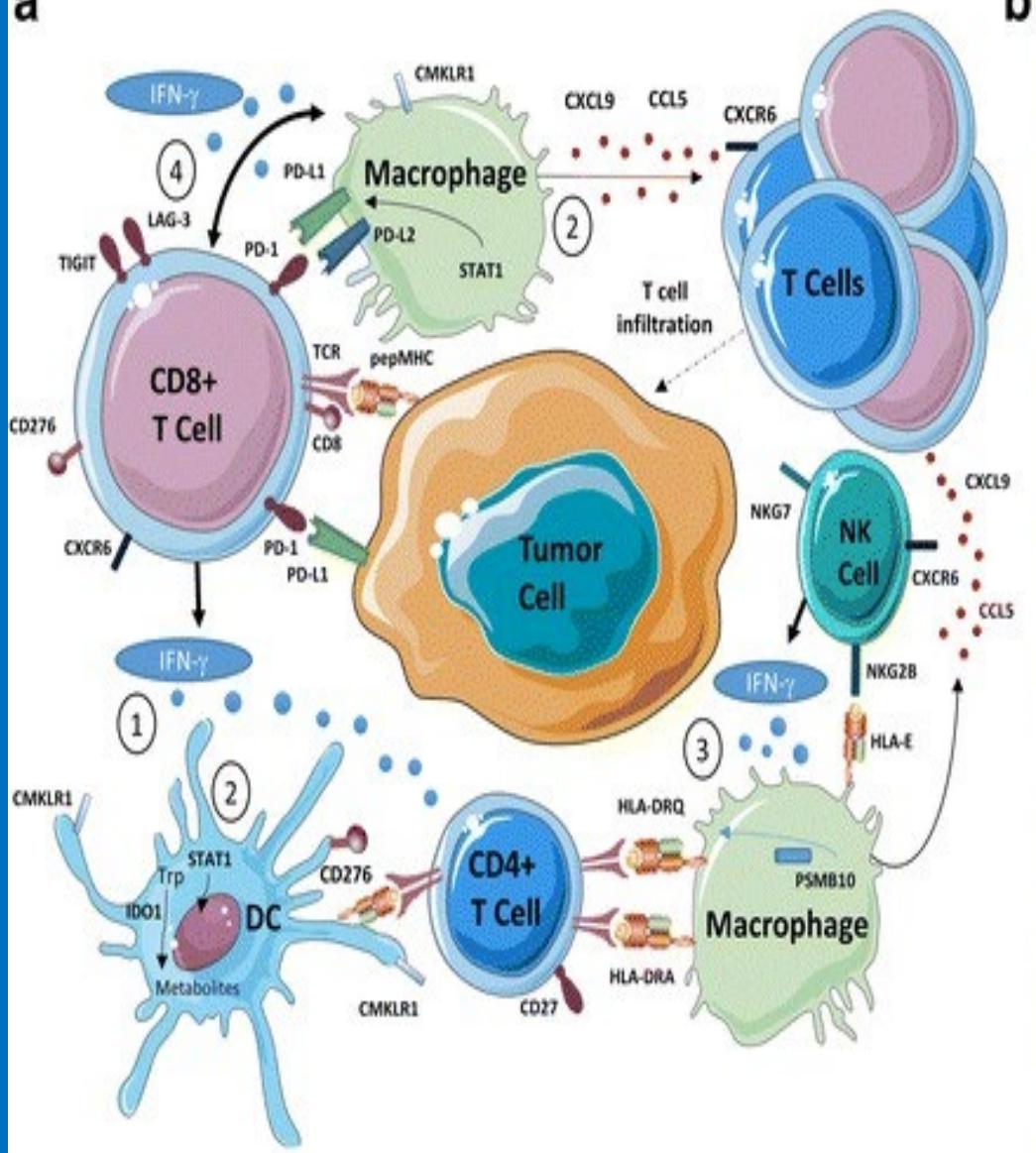


b

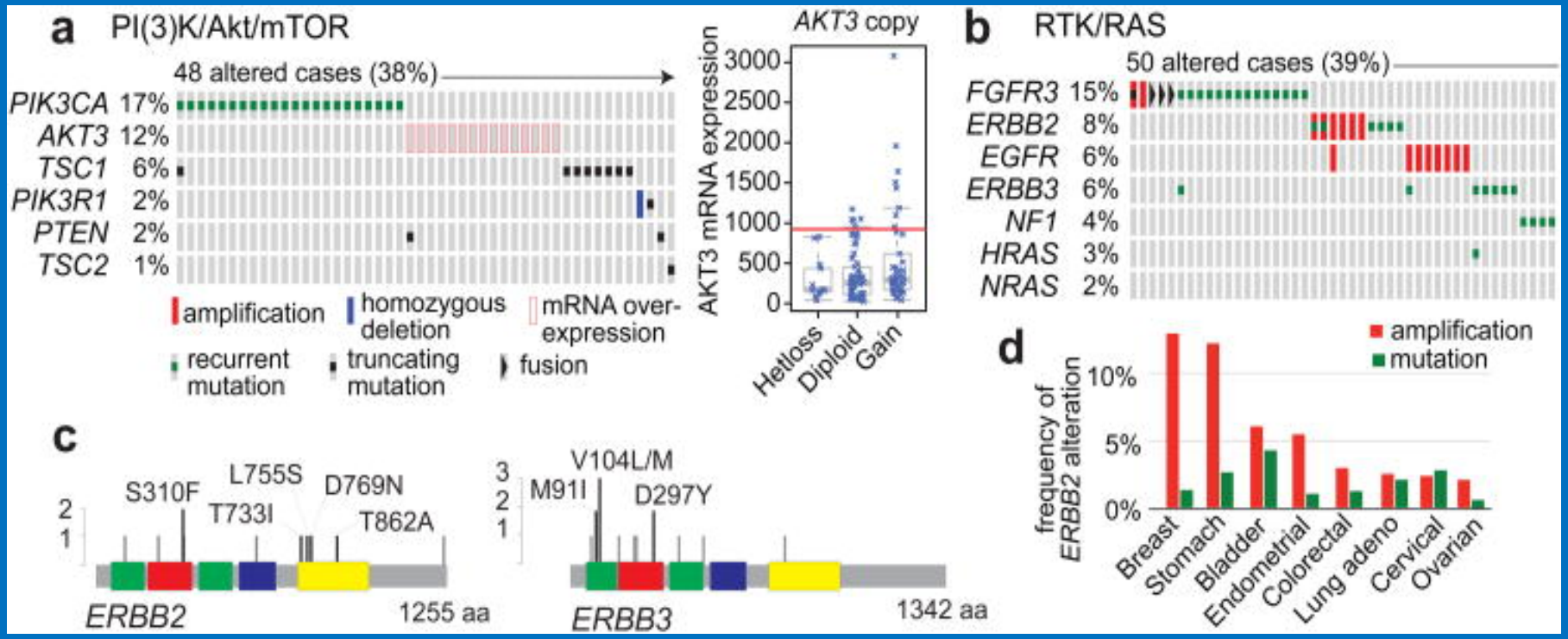
Gene	Immunologic Function
T Cell Exhaustion	
CD8A	T cell coreceptor, heterodimer with the CD8β unit required for TCR:class I MHC interaction
PD-L1	Immune checkpoint upregulated in response to chronic inflammation and IFN-γ; ligation inhibits T cell proliferation/cytokine production
PD-L2	Immune checkpoint upregulated in response to chronic inflammation and IFN-γ; ligation inhibits signal 2 (B7-CD28) and decreases TCR mediation proliferation/cytokine production
LAG-3	Lymphocyte activating gene 3. Binds class II MHC with high affinity and negatively regulates T cell activity
TIGIT	T cell immunoreceptor with Ig and ITIM domains; binds CD155 on DCs and macrophages and suppresses T cell response
CD27	TNF superfamily receptor necessary for maintenance of T cell immunity and B cell activation; binds to CD70
CD276 (B7-H3)	Expressed on APCs and T Cells, inhibits T cell function. Only marker downregulated in IFN-γ 18-gene signature that correlates with anti-PD1 response
Cytokines, Chemokines, and Chemokine Receptors	
CCL5	Chemokine for monocytes, memory T helper cells, and eosinophils
CXCL9	Chemokine for T cells
CXCR6	Chemokine receptor expressed on NK cells, T cells, and plasma cells
IFN-γ Related Proteins	
IDO-1	Enzyme that catalyzes the destructive metabolism of tryptophan; expressed in macrophages, DCs, and monocytes
Antigen Presenting Cell Proteins	
PSMB10	Proteasome subunit 10, forms component of proteasome complex for professional APCs
STAT-1	Transcription factor activated by IFN-γ; promotes increased PD-L1 and PSMB10 expression
HLA-DQA1	Class II MHC
HLA-DRB1	Class II MHC
HLA-E	Class I MHC that presents signal peptides from class I MHC complexes recognized by inhibitory NK cell receptor NKG2B
NK Cell Related Proteins	
NKG-7	Cell surface protein on NK and T cells; upregulated in response to G-CSF
CMKLR1	Chemokine-like receptor 1; receptor for chimerin, a chemoattract for NK cells, macrophages, and DC subsets

- On-going combination immunotherapy trials in urothelial cancer

Therapy	Number	Phase	Trial ID	Est. Completion		
Nivolumab +/− Ipilimumab (CheckMate-032)	1150					
70	I/II					
II	NCT01928394					
NCT02553642	December 2018					
September 2018						
Atezolizumab + MOXR0916 (anti-OX40) +/− Bevacizumab	762	I	NCT02410512	August 2018		
CPI-444 + Atezolizumab	534	I/Ib	NCT02655822	June 2018		
Pembrolizumab + PLX3397 (CSF1R)	400	I/II	NCT02452424	July 2019		
BMS-986106 (anti-LAG3) +/− Nivolumab	360	I/II	NCT01968109	October 2019		
MK-7684 +/− Pembrolizumab	336	I	NCT02964013	March 2020		
GSK3174998 (anti-OX40) +/− Pembrolizumab	264	I	NCT02528357	January 2020		
Pembrolizuamb + Lenvatinib	250	Ib/II	NCT02501096	January 2018		
Durvalumab + Epacadostat	185	I/II	NCT02318277	January 2018		
Pembrolizumab + Ramucirumab	155	I	NCT02443324	June 2018		
Nivolumab + Cabozantinib +/− Ipilimumab	135	I	NCT02496208	December 2017		
Atezolizumab + Epacadostat	118	I	NCT02298153	November 2020		
Durvalumab + (Olaparib or Vistusertib or						
AZD1775 or AZD4547)	110	I	NCT02546661	March 2019		
Durvalumab + Tremelimumab + polyICLC (TLR3 agonist)	102	I/II	NCT02643303	August 2022		
Pembrolizuamb +/− Acalabrutinib	75	II	NCT02351739	Summer 2017		
Tremelimumab +/− Durvalumab	64	II	NCT02527434	October 2018		
Ipilimumab + Enoblituzumab (anti-B7-H3)	59	I	NCT02381314	March 2018		
Atezolizumab + B-701 (FGFR3 inhibitor)	48	Ib	NCT03123055	April 2017		
Pembrolizumab + Vorinostat	42	I	NCT02619253	May 2018		
Pembrolizumab + Docetaxel or Gemcitabine	38	I	NCT02437370	December 2019		
Nivolumab + Enadenotucirev (oncolytic virus)	30	I	NCT02636036	June 2018		
Avelumab + NHS-IL-12	30	I	NCT02994953	April 2018		
Pembrolizumab + Paclitaxel	27	II	NCT02581982	March 2019		
Nivolumab + IFN-γ	15	I	NCT02614456	December 2017		

On-going studies evaluating first-line therapies for metastatic urothelial cancer

Therapy	Number	Phase	Primary Endpoint	Trial ID	Estimated Completion Date
Atezolizumab + Gemcitabine/Carboplatin vs. Gemcitabine/Carboplatin (IMvigor 130)	1200	III	PFS/OS	NCT02807636	July 2020
Pembrolizumab +/− Platinum vs Gemcitabine/Platinum (Keynote 361)	990	III	PFS/OS	NCT02853305	March 2020
Durvalumab +/− Tremelimumab vs. Gemcitabine/Carboplatin (1:1:1)	525	III	PFS/OS	NCT02516241	July 2019
Pembrolizumab + CVA21 (Coxsackievirus A21)	90	I	Safety	NCT02043665	August 2019
Nivolumab + NEO-PV-1 (personalized peptide vaccine)	90	Ib	Safety	NCT02897765	December 2020
Pembrolizumab + sEphB4-HSA	60	II	OS	NCT02717156	November 2020
Gemcitabine/Cisplatin +/− Ipilimumab (Active, not accruing)	36	II	Safety/ORR	NCT01524991	November 2017
Atezolizumab +/− Gemcitabine Cisplatin (First line metastatic or MIBC)	30	I/II	Safety	NCT02989584	December 2020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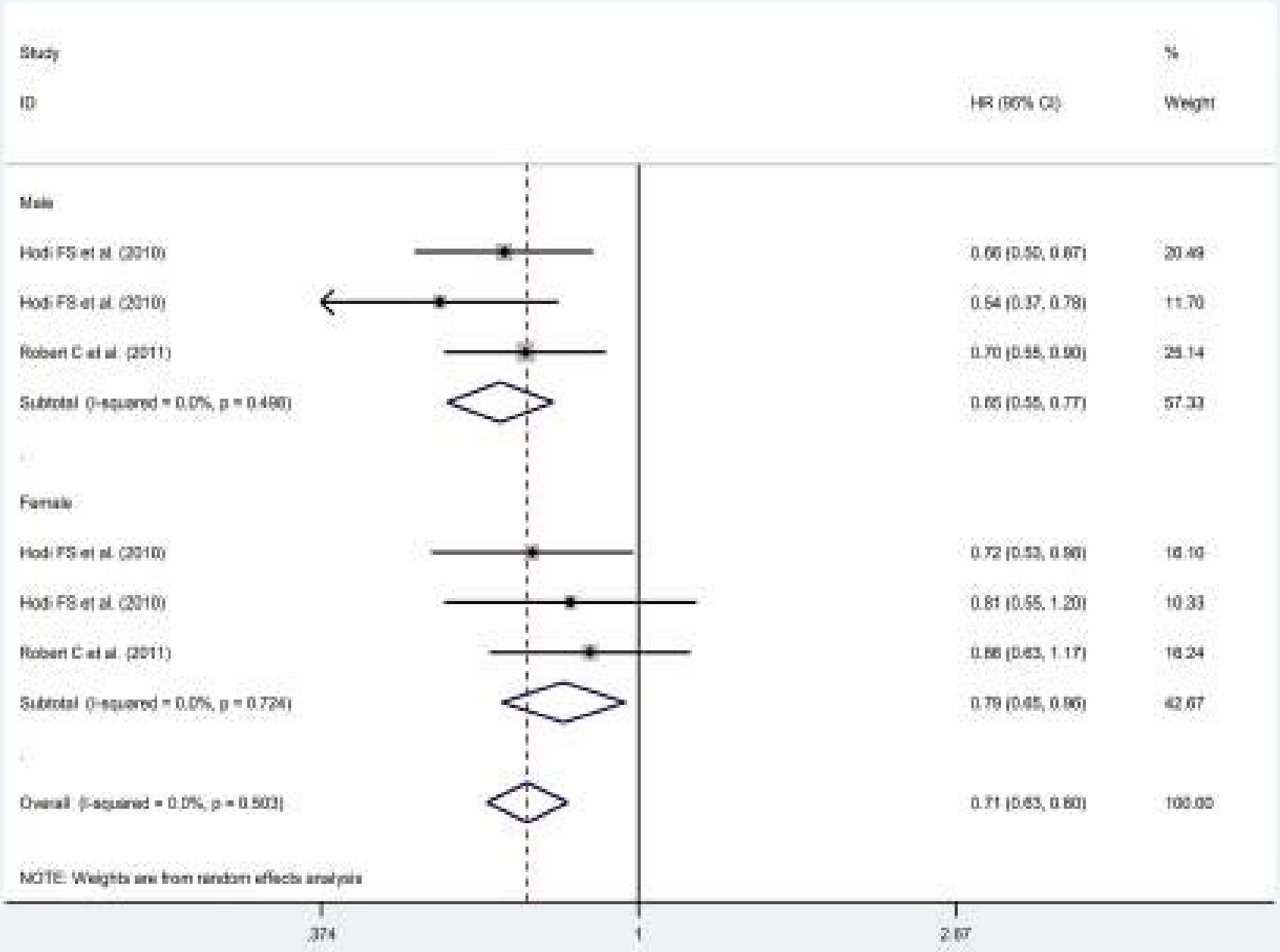
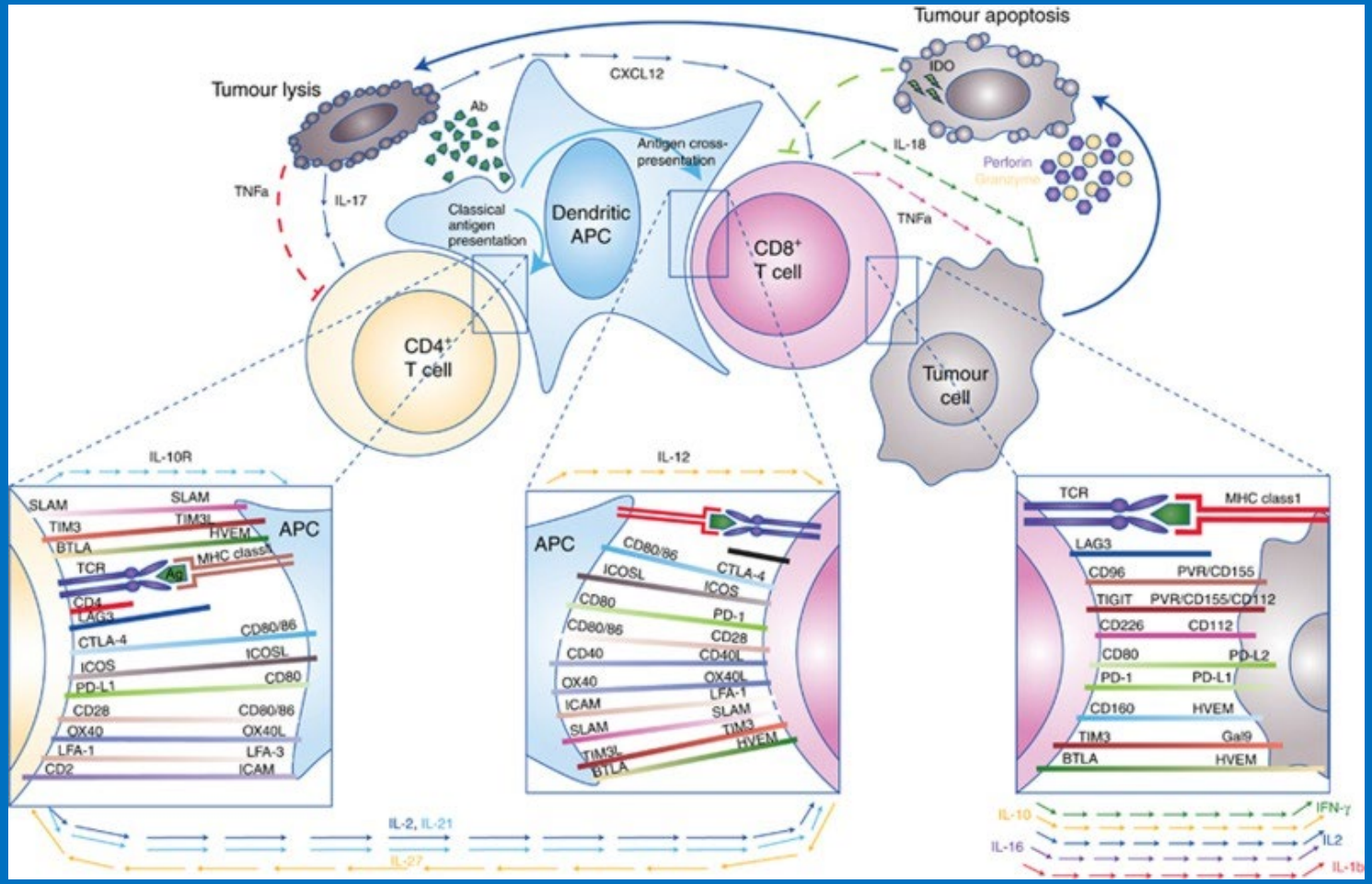
PI(3)K/Akt/mTOR
48 altered cases (38%)
PIK3CA 17%
AKT3 12%
TSC1 6%
PIK3R1 2%
PTEN 2%
TSC2 1%
amplification
homozygous deletion
mRNA over-expression
recurrent mutation
truncating mutation
fusion
AKT3 copy
AKT3 mRNA expression
3000
2500
2000
1500
1000
500
0
Hetloss
Diploid
Gain
b
RTK/RAS
50 altered cases (39%)
FGFR3 15%
ERBB2 8%
EGFR 6%
ERBB3 6%
NF1 4%
HRAS 3%
NRAS 2%
c
S310F
L755S
D769N
T733I
T862A
ERBB2
1255 aa
V104L/M
M91I
D297Y
ERBB3
1342 aa
d
amplification
mutation
frequency of ERBB2 alteration
10%
5%
0%
Breast
Stomach
Bladder
Endometrial
Colorectal
Lung adeno
Cervical
Ovarian

Clinical trials selected for OS

Author/year	Clinical trial	Cancer	Treatment	N pts	M	F	OS HR	OS M HR	OS M range	OS F HR	OS F range
Hodi et al. 2010 [13]	Phase III	Melanoma St. III, IV	Ipilimumab + Gp100	403	247	156	0.68	0.66	0.50-0.87	0.72	0.52-0.99
			Ipilimumab	137	81	56	0.66	0.54	0.37-0.77	0.81	0.55-1.20
			Gp100	136	73	63					
Robert et al. 2011 [16]	Phase III	Melanoma St. IV	Ipilimumab + Dacarbazina	250	152	98	0.72	-0.35*	-0.60- -0.1*	-0.15*	-0.46- -0.16*
			Dacarbazina	252	149	103					
Motzer et al. 2015 [29]	Phase III	Kidney St. IV	Nivolumab 3 mg/kg	410	315	95	0.73(0.57-0.93)	0.73	0.58-0.92	0.84	0.57-1.24
			Eveolimus	411	304	107					
Bellmont et al. 2017 [30]	Phase III	Urothelial St. IV	Pembrolizumab 200 mg q21	270	200	70	0.73 (0.59-0.91)	0.73	0.56-0.94	0.78	0.49-1.24
			Chemotherapy	272	202	70					
Brahmer et al. 2015 [26]	Phase III	NSCLC St. IIIB/IV	Nivolumab 3 mg/kg	135	11	24	0.59(0.44-0.79)	0.57	0.41-0.78	0.67	0.36-1.25
			Docetaxel	137	97	40					
Borghaei et al. 2015 [27]	Phase III	NSCLC ADK St. IIIB/IV	Nivolumab 3 mg/kg	292	151	141	0.73 (0.59-0.89)	0.73	0.56-0.96	0.78	0.58-1.04
			Docetaxel	290	168	122					
Carbone et al. 2017 [28]	Phase III	NSCLC St. IV/recurent	Nivolumab 3 mg/kg	271	184	87	1.02 (0.80-1.30)	0.87	0.74-1-.26	1.15	0.79-1.66
			Chemotherapy	270	148	122					
Herbst et al. 2016 [22]	Phase II/III	NCSLC St. IV	Pembrolizumab 2 mg/kg	345	212	133	0.71(0.58-0.88)	0.65	0.52-0.81	0.69	0.51-0.94
			Pembrolizumab 10 mg/kg	346	213	133	0.61 (0.49-0.75)				
			Docetaxel	343	209	134					
Rittmeyer et al. 2017 [25]	Phase III	NSCLC St. IIIB/IV	Atezolizumab 1200 mg	425	261	164	0.73 (0.62-0.87)	0.79	0.64-0.97	0.64	0.49-0.85
			Docetaxel	425	259	166					


Study
ID
HR (95% CI)
%
Weight
Male
Hodi FS et al. (2010)
0.66 (0.50, 0.87)
20.49
Hodi FS et al. (2010)
0.54 (0.37, 0.78)
11.70
Robert C et al. (2011)
0.70 (0.55, 0.90)
25.14
Subtotal (I-squared = 0.0%, p = 0.490)
0.65 (0.55, 0.77)
57.33
Female
Hodi FS et al. (2010)
0.72 (0.53, 0.98)
18.10
Hodi FS et al. (2010)
0.81 (0.55, 1.20)
10.33
Robert C et al. (2011)
0.88 (0.63, 1.17)
16.24
Subtotal (I-squared = 0.0%, p = 0.724)
0.79 (0.65, 0.96)
42.67
Overall (I-squared = 0.0%, p = 0.503)
0.71 (0.63, 0.80)
100.00
NOTE: Weights are from random effects analysis
.374
1
2.67


Tumour apoptosis
Tumour lysis
CXCL12
IDO
Ab
Antigen cross-presentation
IL-18
Perforin
Granzyme
TNFa
IL-17
Classical antigen presentation
Dendritic APC
CD8+ T cell
TNFa
CD4+ T cell
Tumour cell
IL-10R
IL-12
SLAM
TIM3
BTLA
TCR
MHC class1
CD4
LAG3
CTLA-4
CD80/86
ICOS
ICOSL
PD-L1
CD80
CD28
OX40
OX40L
LFA-1
LFA-3
CD2
ICAM
APC
TIM3L
HVEM
CD40
CD40L
SLAM
BTLA
PVR/CD155
CD96
TIGIT
PVR/CD155/CD112
CD226
CD112
PD-L2
PD-1
PD-L1
CD160
Gal9
IL-2, IL-21
IL-27
IL-10
IL-16
IFN-γ
IL2
IL-1b

Neoplasie uroteliali