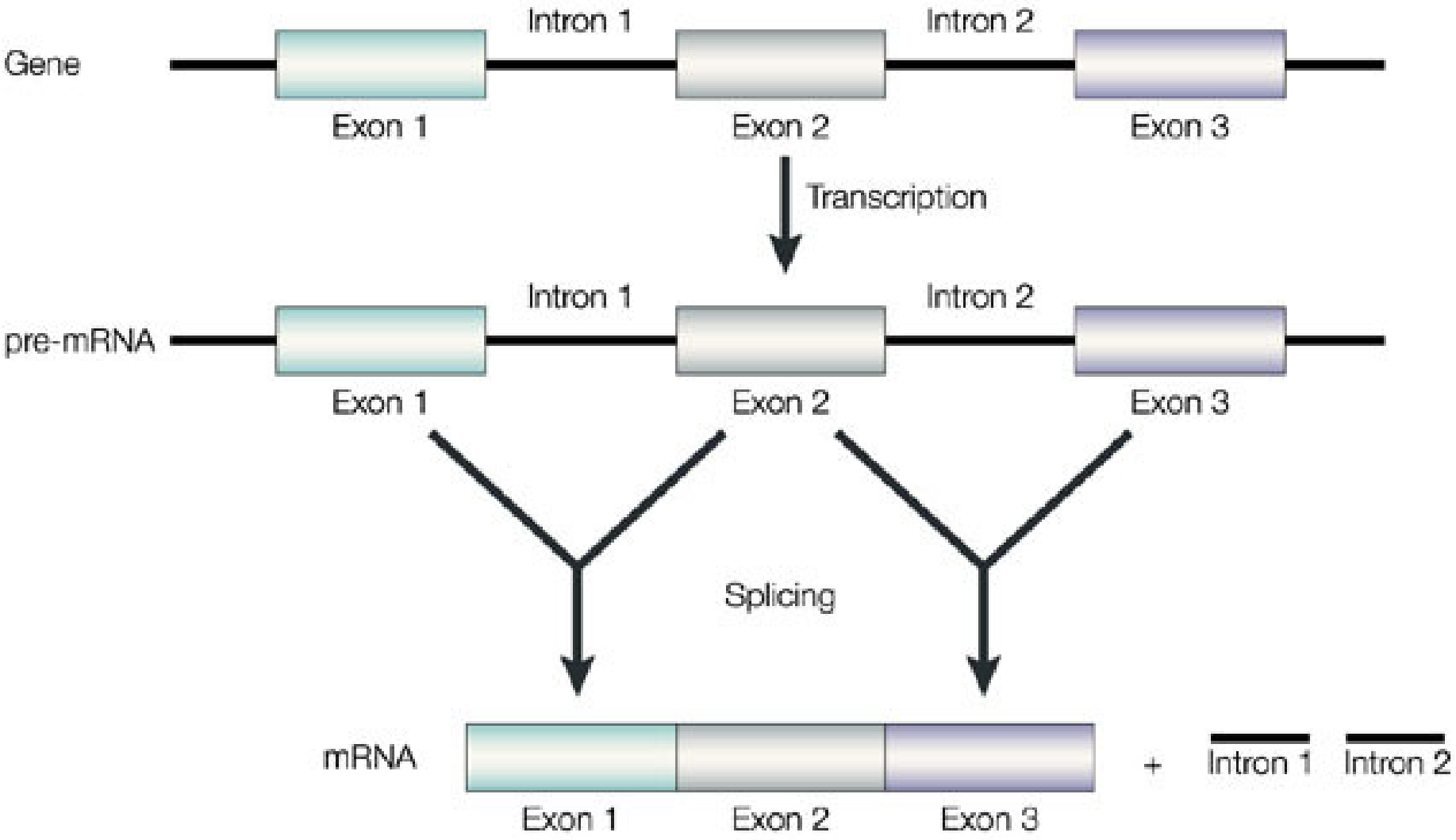
Splicing variants.

P. Pauwels
(UZA, UA)

Kennis / Ervaring / Zorg


Gene
Intron 1
Intron 2
Exon 1
Exon 2
Exon 3
Transcription
pre-mRNA
Intron 1
Intron 2
Exon 1
Exon 2
Exon 3
Splicing
mRNA
Exon 1
Exon 2
Exon 3
+
Intron 1
Intron 2

OXFORD

JNCI J Natl Cancer Inst (2017) 109(5): djw262

doi: 10.1093/jnci/djw262
First published online February 10, 2017
Review

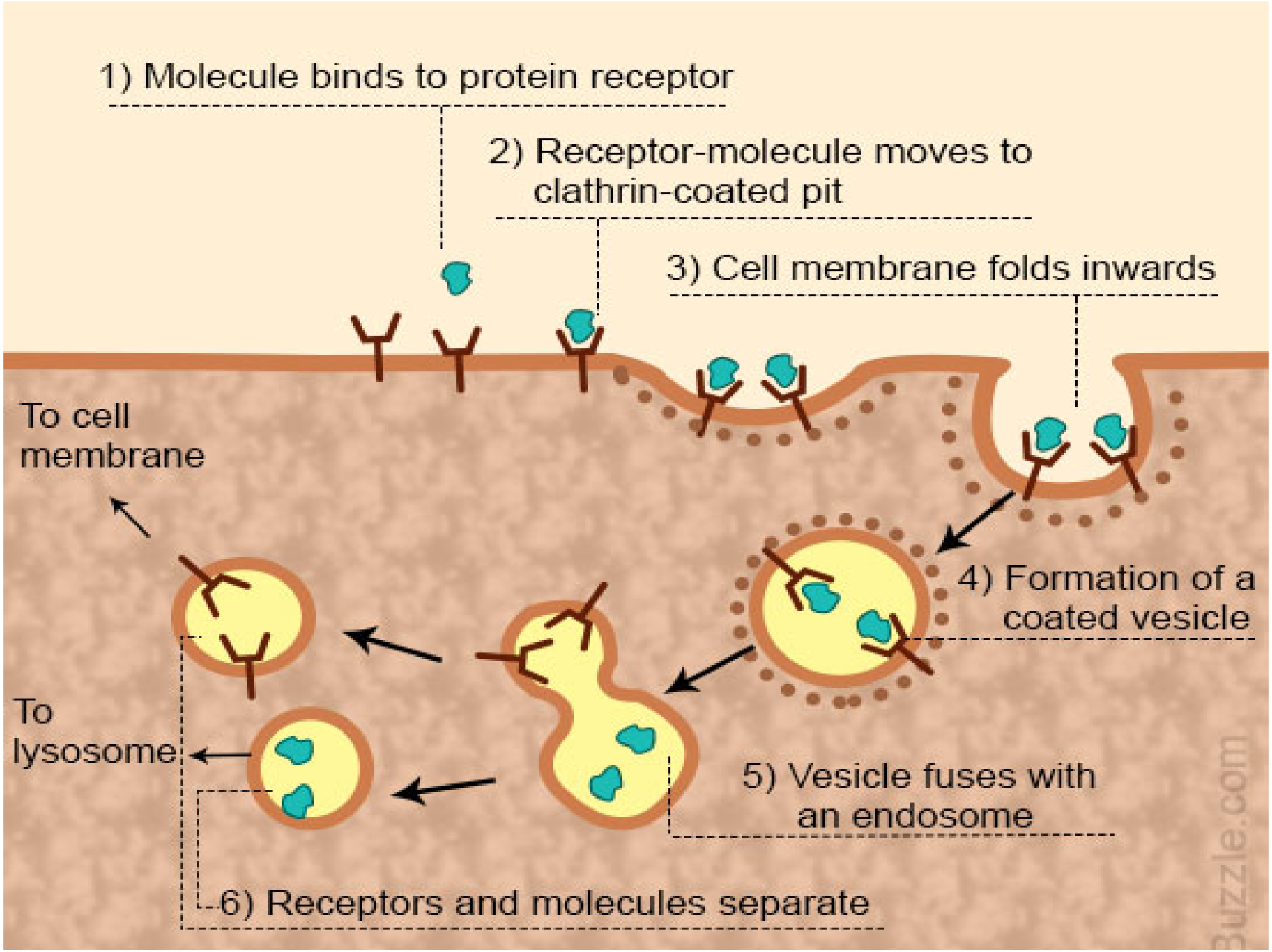
REVIEW

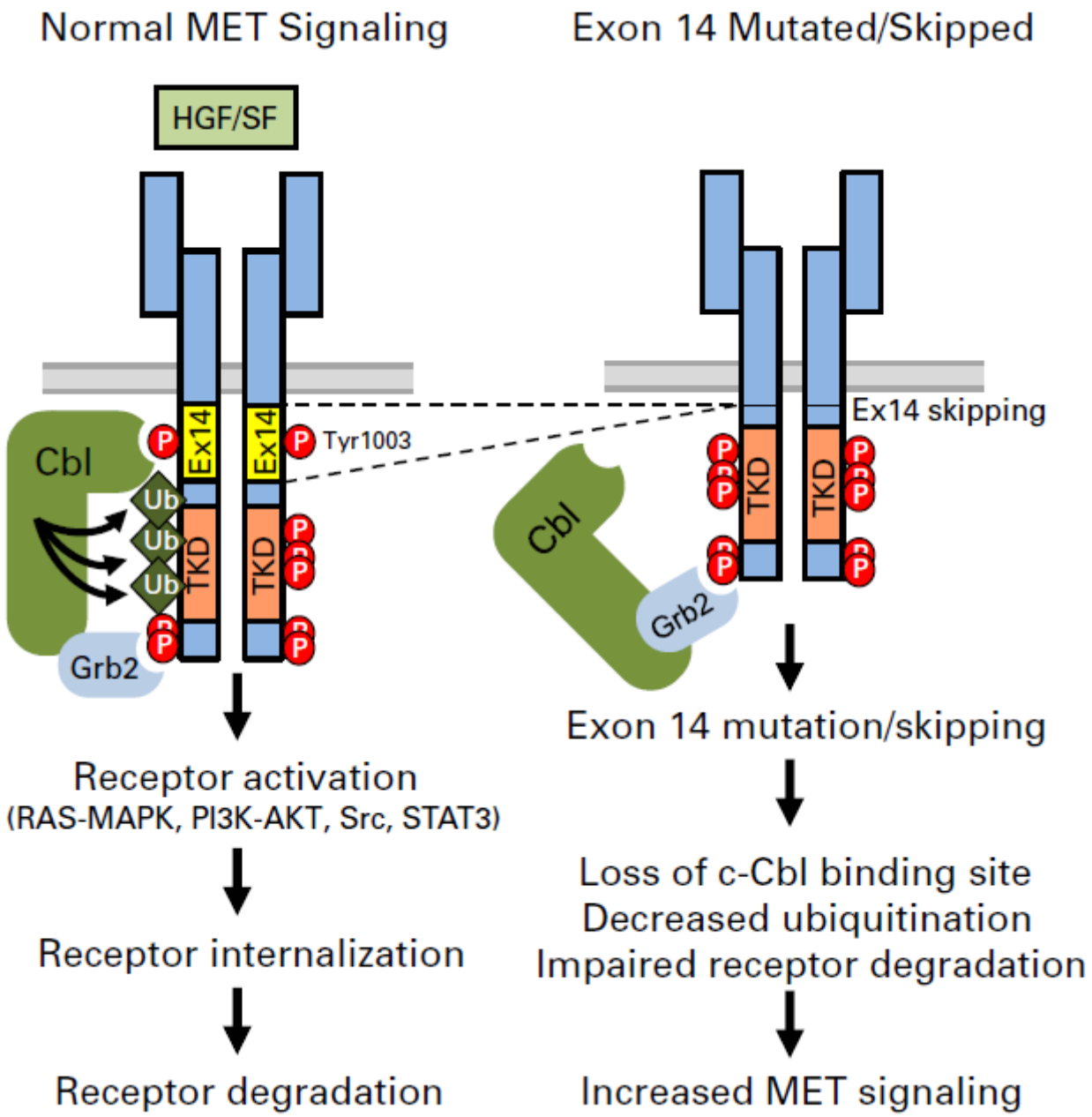
Exon 14 Deleted MET Receptor as a New Biomarker and Target in Cancers

Alexis B. Cortot, Zoulika Kherrouche, Clotilde Descarpentries, Marie Wislez, Simon Baldacci, Alessandro Furlan, David Tulasne

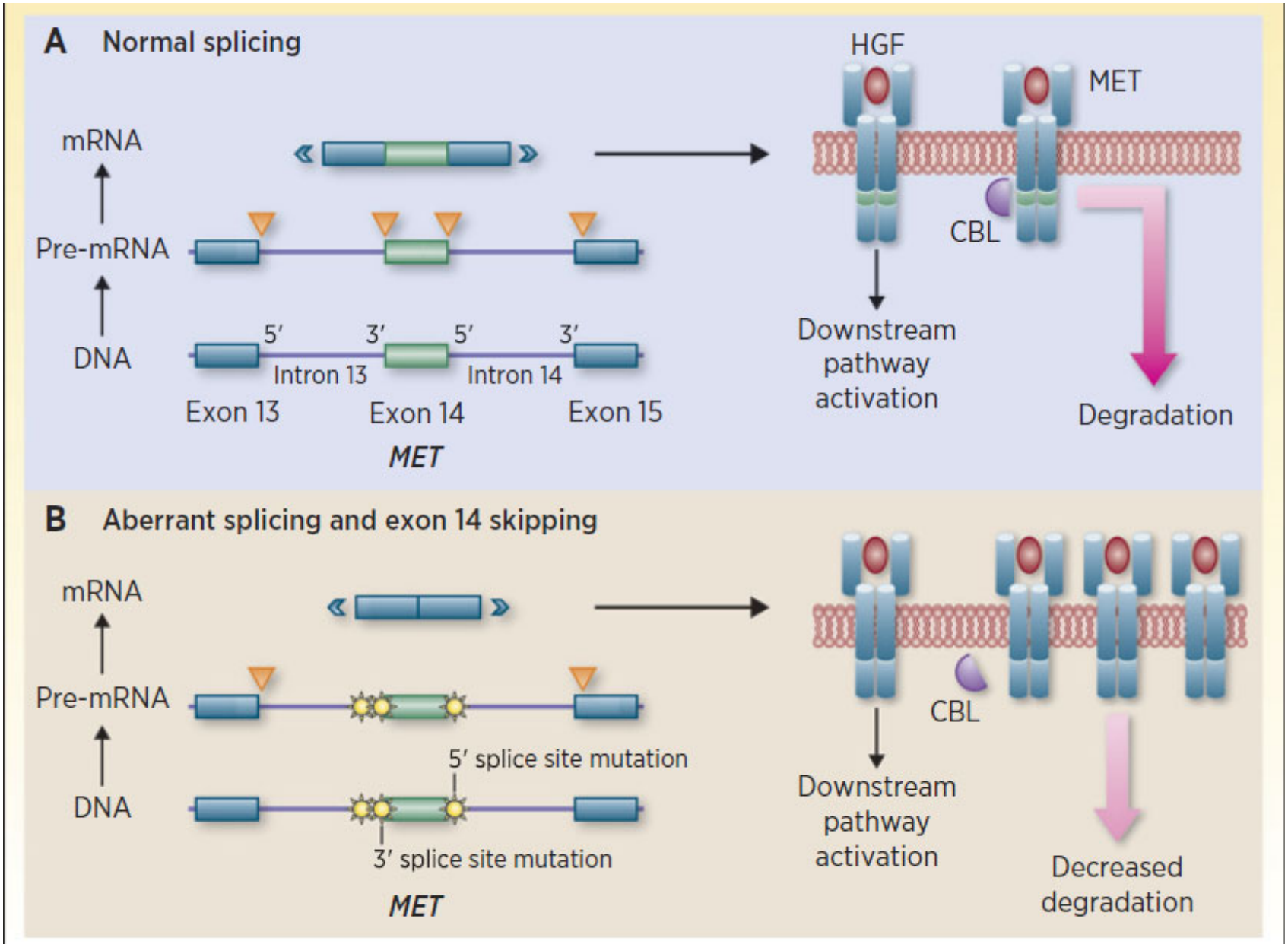
Affiliations of authors: UMR 8161 - M3T - Mechanisms of Tumorigenesis and Targeted Therapies, CNRS, Institut Pasteur de Lille, Univ. Lille, Lille, France (ABC, ZK, SB, AF, DT); Thoracic Oncology Department, CHU Lille, Univ. Lille, Lille, France (ABC); Division of Biochemistry and Molecular Biology, Oncology and Molecular Genetics Laboratory, CHU Lille, Lille, France (CD); Service de Pneumologie, Hôpital Tenon, AP-HP, Paris, France (MW).

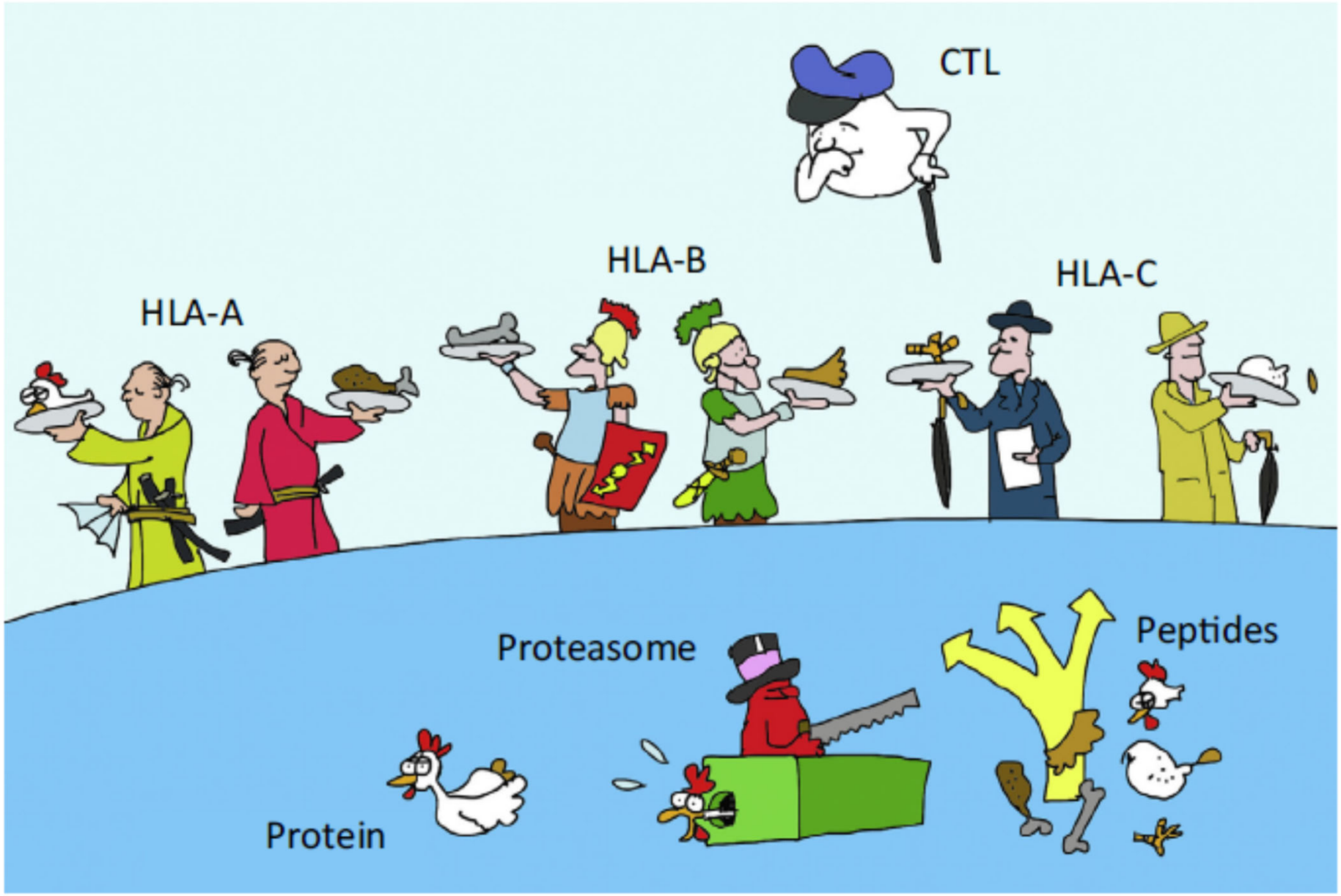
Correspondence to: David Tulasne, PhD, UMR 8161 - M3T - Mechanisms of Tumorigenesis and Targeted Therapies, CNRS, Institut Pasteur de Lille, Univ. Lille, F-59000 Lille, France (e-mail: david.tulasne@ibl.cnrs.fr); or Alexis B. Cortot, MD, PhD, UMR 8161 - M3T - Mechanisms of Tumorigenesis and Targeted Therapies, CNRS, Institut Pasteur de Lille, Univ. Lille, F-59000 Lille, France (e-mail: alexis.cortot@chru-lille.fr).


1) Molecule binds to protein receptor
2) Receptor-molecule moves to clathrin-coated pit
3) Cell membrane folds inwards
To cell membrane
4) Formation of a coated vesicle
To lysosome
5) Vesicle fuses with an endosome
6) Receptors and molecules separate
Buzzle.com

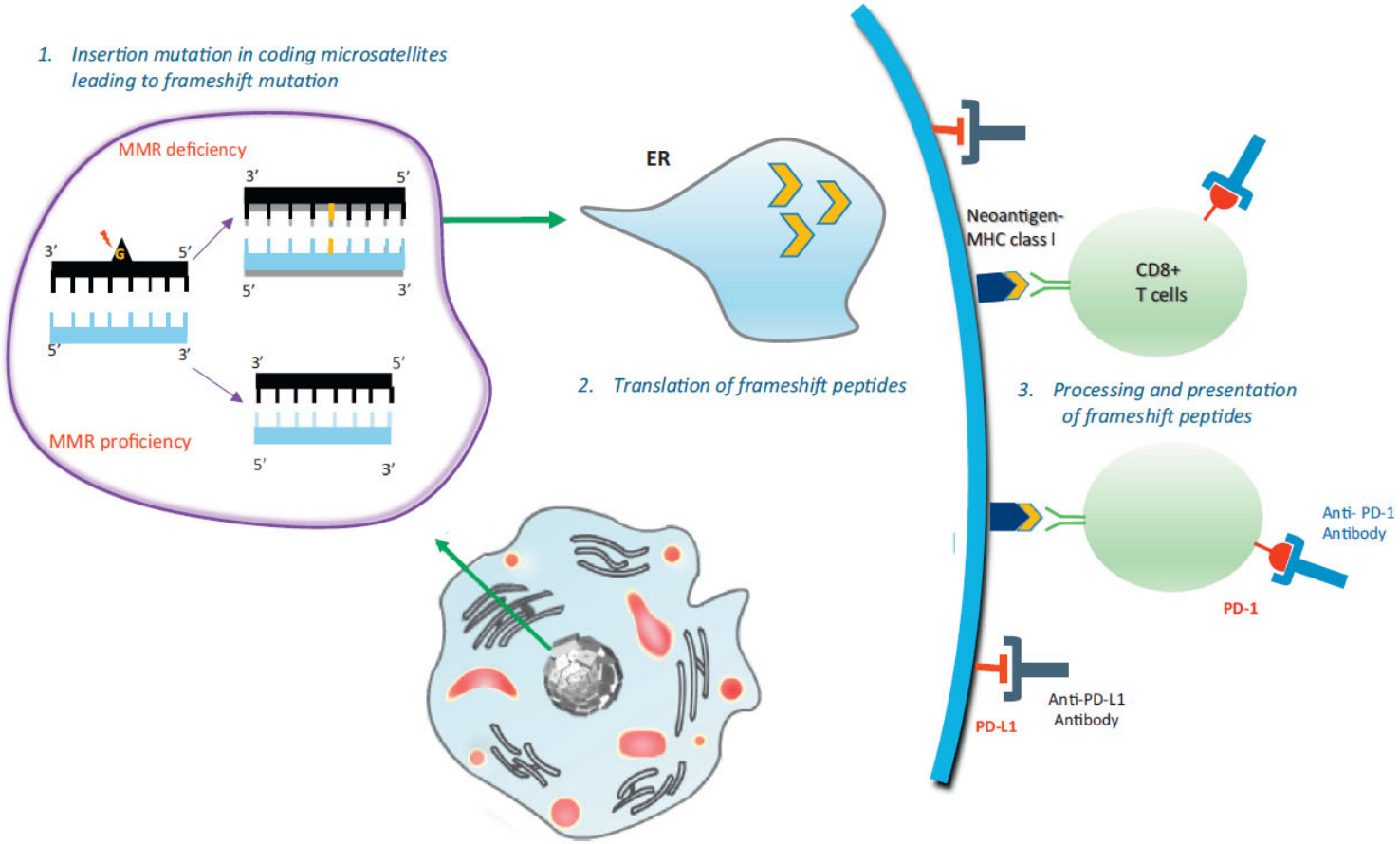


Journal of Clinical Oncology, Vol 34, No 8 (March 10), 2016: pp 879-881

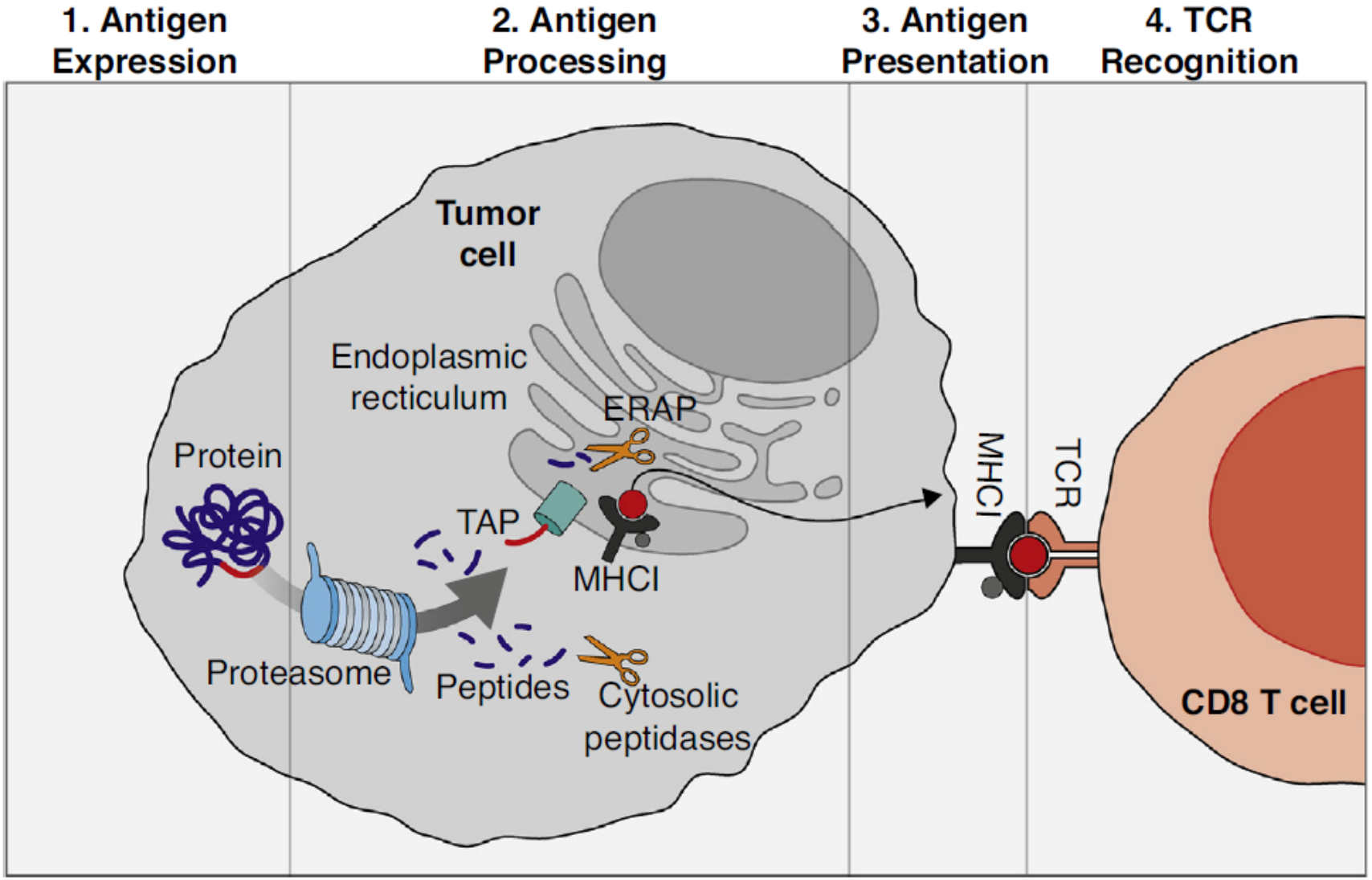




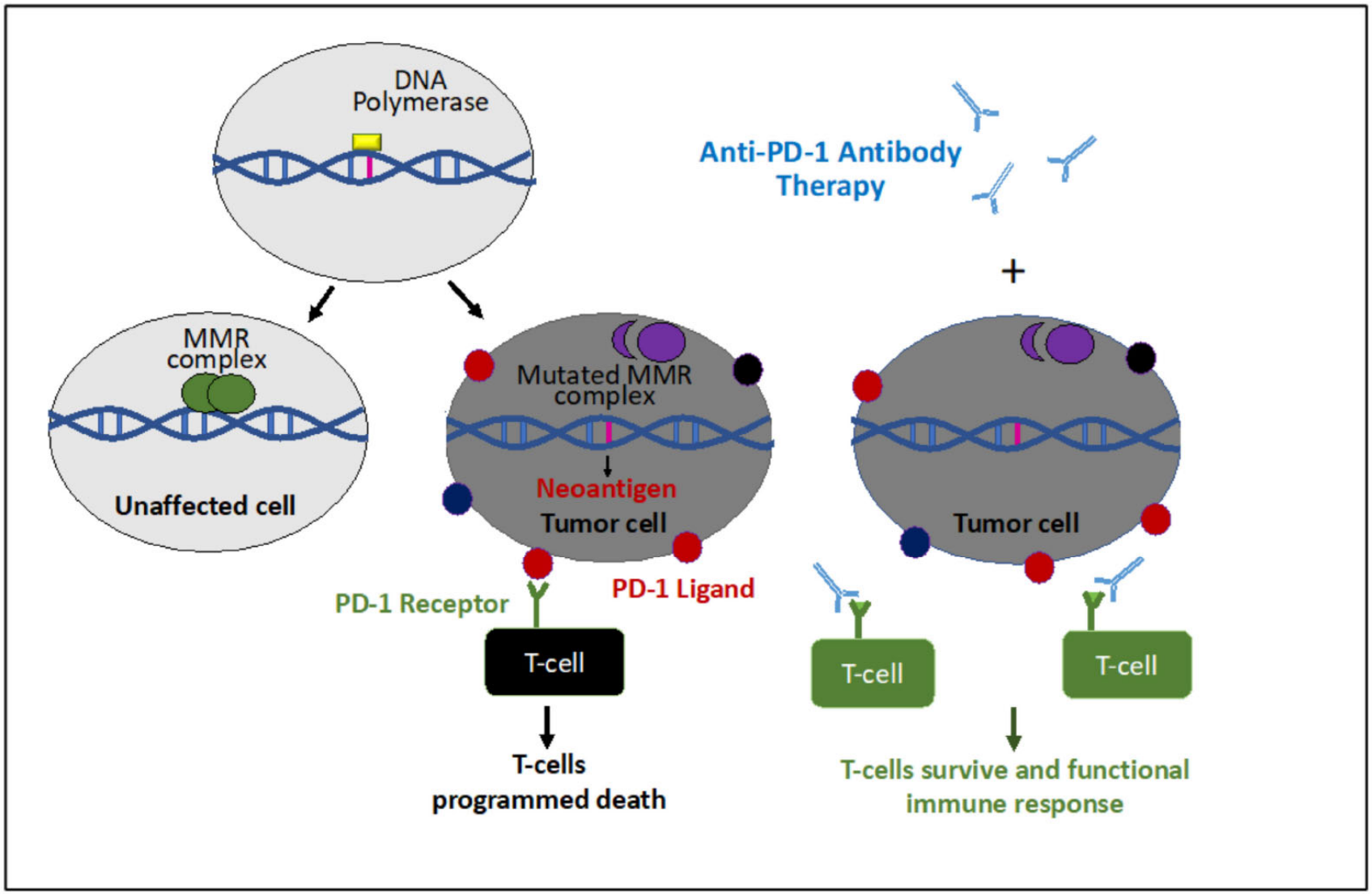
Trends in Immunology



M. Baretti, D.T. Le / Pharmacology & Therapeutics 189 (2018) 45–62



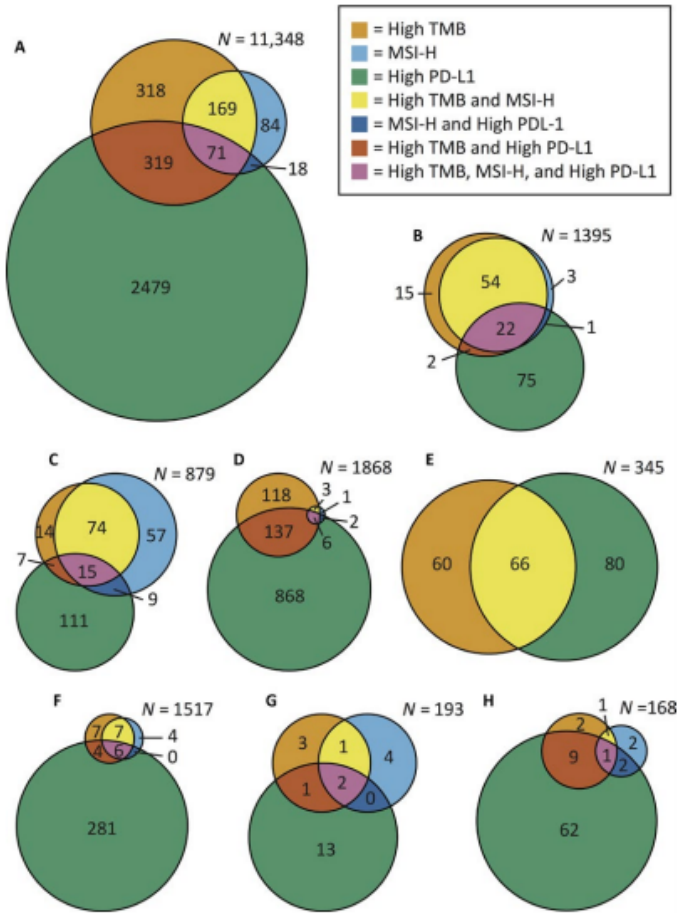
Current Opinion in Immunology


DNA
Polymerase
MMR
complex
Unaffected cell
Mutated MMR
complex
Neoantigen
Tumor cell
PD-1 Receptor
PD-1 Ligand
T-cell
T-cells
programmed death
Anti-PD-1 Antibody
Therapy
+
Tumor cell
T-cell
T-cell
T-cells survive and functional
immune response

Tumor Mutational Burden (TMB) or Tumor Mutation Load (TML)

TMB or TML: total number of somatic/acquired mutations per coding area of a tumor genome (Mut/Mb)

The number of mutations can vary across different tumor types.



doi: 10.1002/cam4.1372

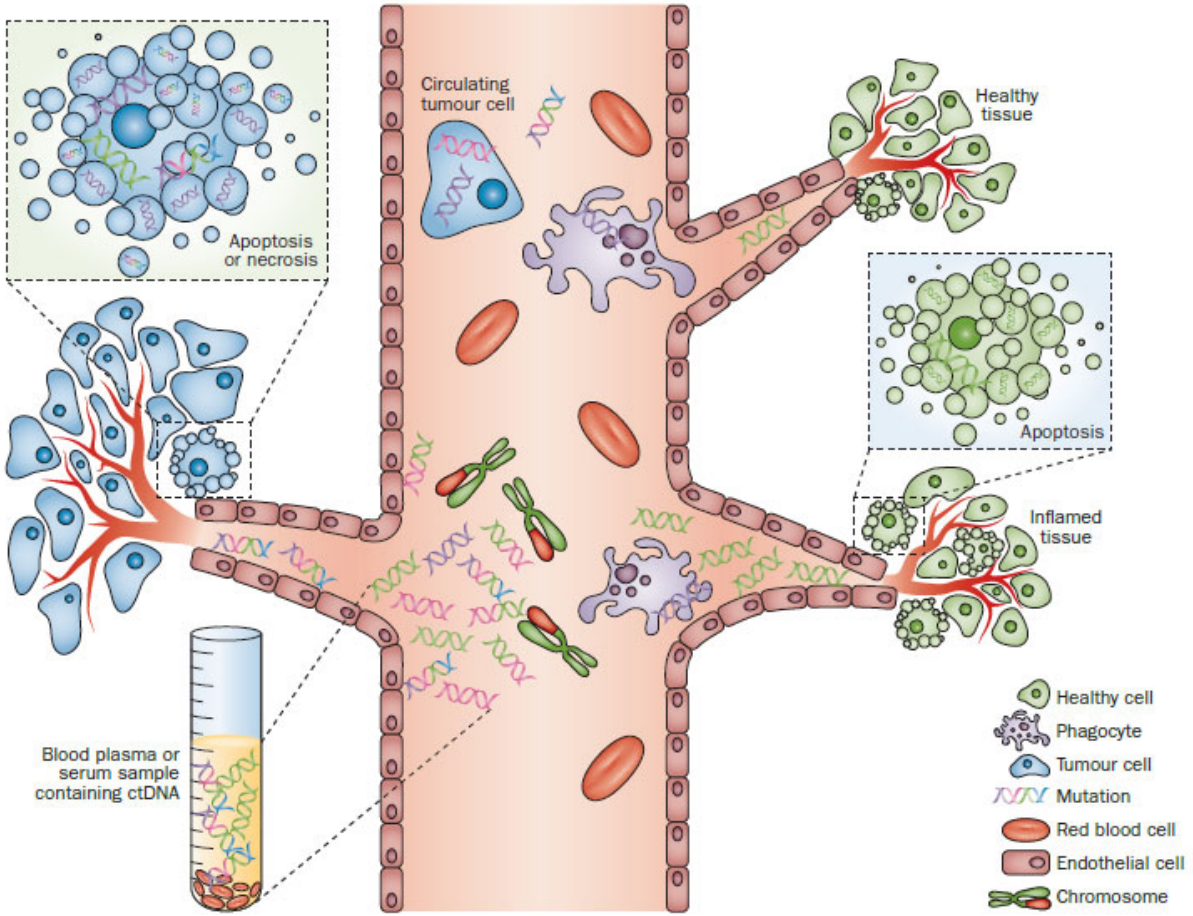
THANK YOU!!

Liquid biopsy: an introduction.

Prof. Dr. P. Pauwels

(UZA, UA)

Kennis / Ervaring / Zorg



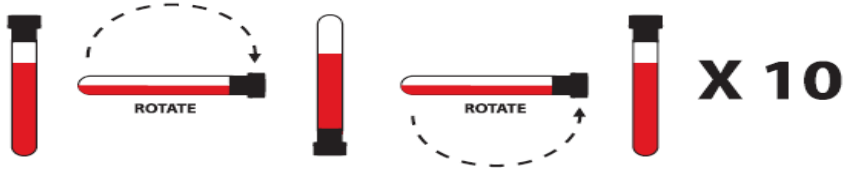
Crowley, E. *et al. Nat. Rev. Clin. Oncol.* **10**, 472–484 (2013)

Liquid Biopsy - practical

Follow up NSCLC patients – # time points

1. **Obtaining a blood sample** (10 ml – STRECK)

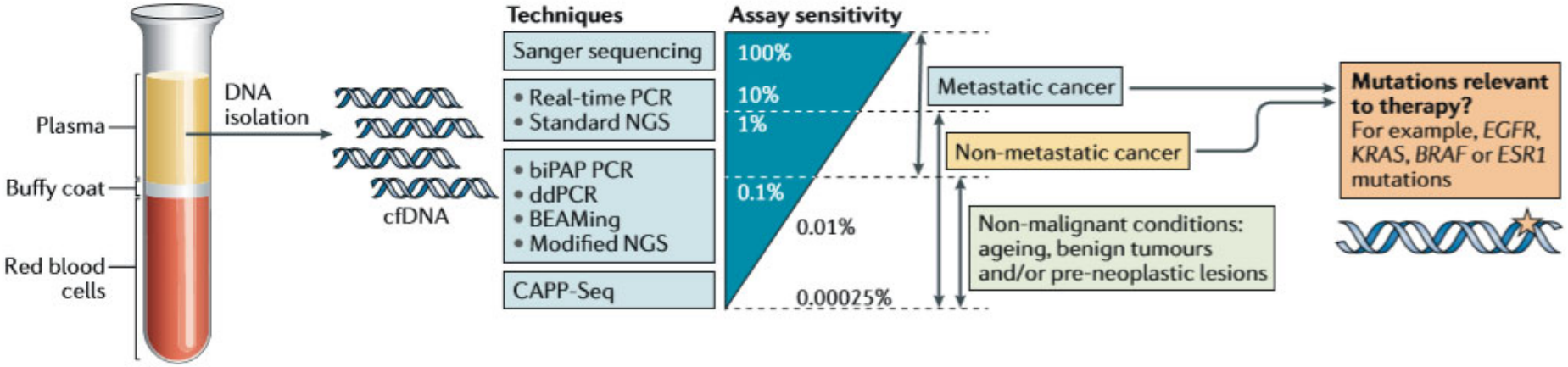
   → Blood: immediately 8 – 10 inversions



Storage & transport at ROOM TEMPERATURE (not in refrigerator)

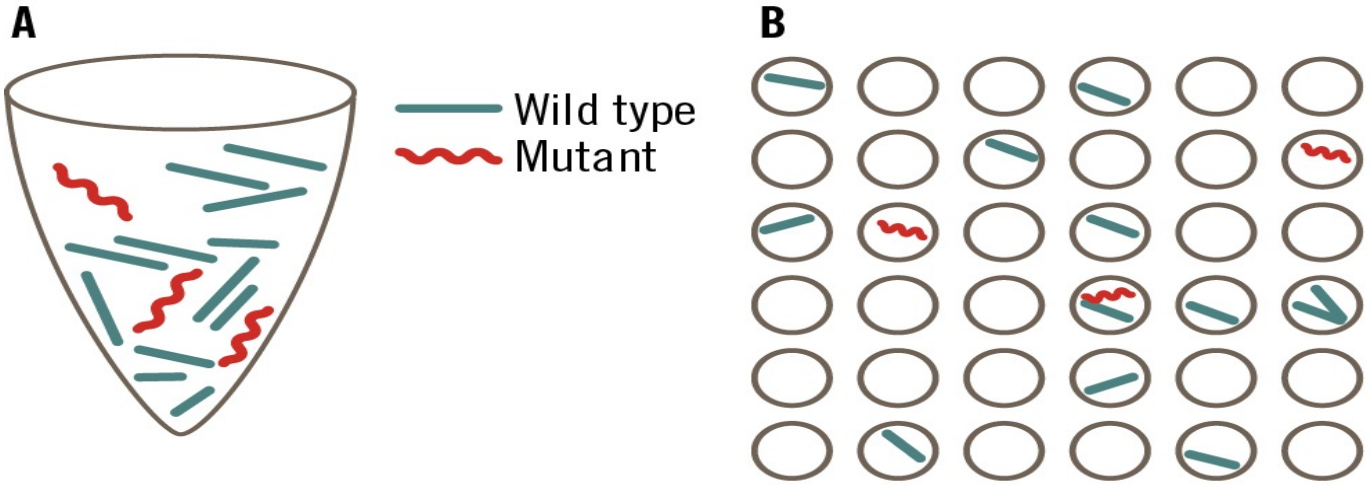
2. **cfDNA extraction** (the MaxWell® ccfDNA Plasma Kit (Promega))

3. **ctDNA detection** by digital droplet PCR (ddPCR)


Plasma
Buffy coat
Red blood cells
DNA isolation
cfDNA
Techniques
Sanger sequencing
• Real-time PCR
• Standard NGS
• biPAP PCR
• ddPCR
• BEAMing
• Modified NGS
CAPP-Seq
Assay sensitivity
100%
10%
1%
0.1%
0.01%
0.00025%
Metastatic cancer
Non-metastatic cancer
Non-malignant conditions: ageing, benign tumours and/or pre-neoplastic lesions
Mutations relevant to therapy?
For example, EGFR, KRAS, BRAF or ESR1 mutations

Liquid Biopsy: ctDNA

Digital Droplet PCR (ddPCR)



Advantages ddPCR

- High sensitivity
- Multiplexing capacity
- Absolute quantification

- Detection: gene amplification, deletion, translocations & gene mutations

Lung Cancer xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

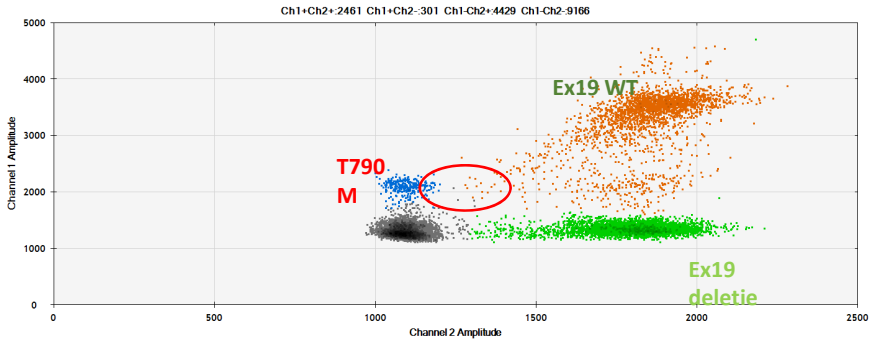
Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Circulating cell-free nucleic acids and platelets as a liquid biopsy in the provision of personalized therapy for lung cancer patients

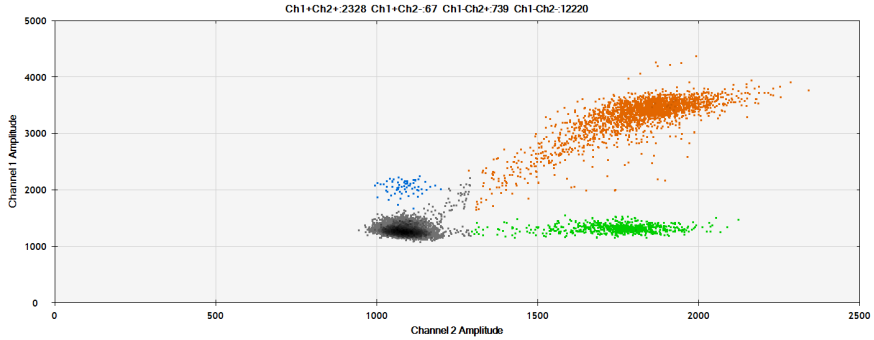
L. Sorber[a,b,*], K. Zwaenepoel[a,b], V. Deschoolmeester[a,b], P.E.Y. Van Schil[c], J. Van Meerbeeck[a,d], F. Lardon[a], C. Rolfo[e], P. Pauwels[a,b]

Patiënt: NSCLC - EGFR exon19 deletie + T790M mutatie



11/02/2015

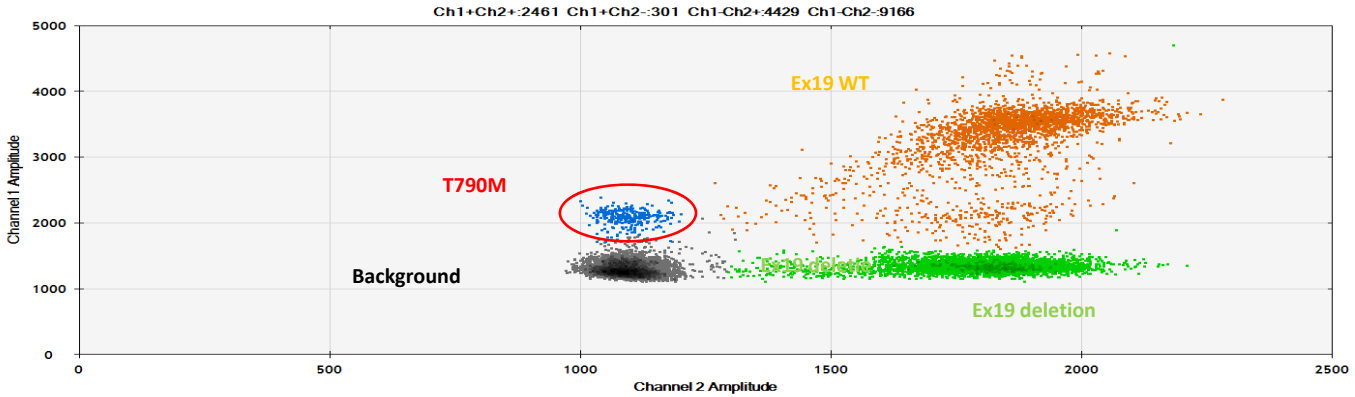
- Geen behandeling
- Ex19 del: 70% - T790M: 3.59%



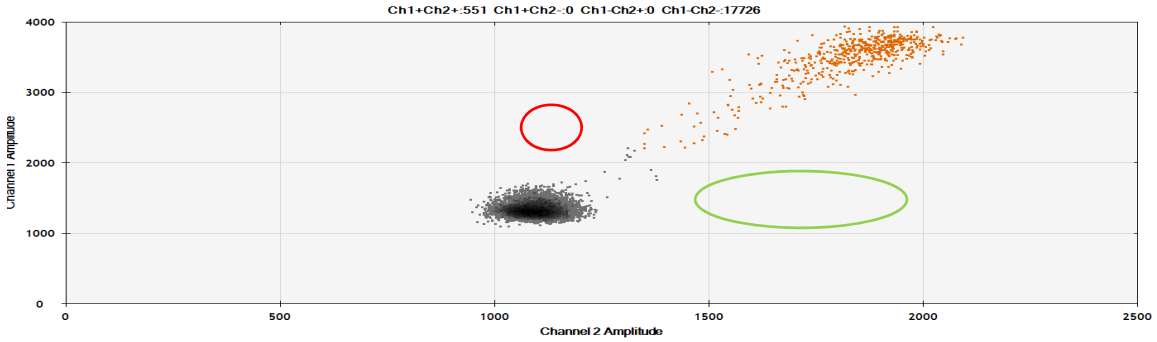
12/03/2015

- Behandeling AZD9291
  (start 06/03/2015)
- Ex19 del: 26.2% - T790M: 1.96%

Patient with EGFR exon 19 del – T790M



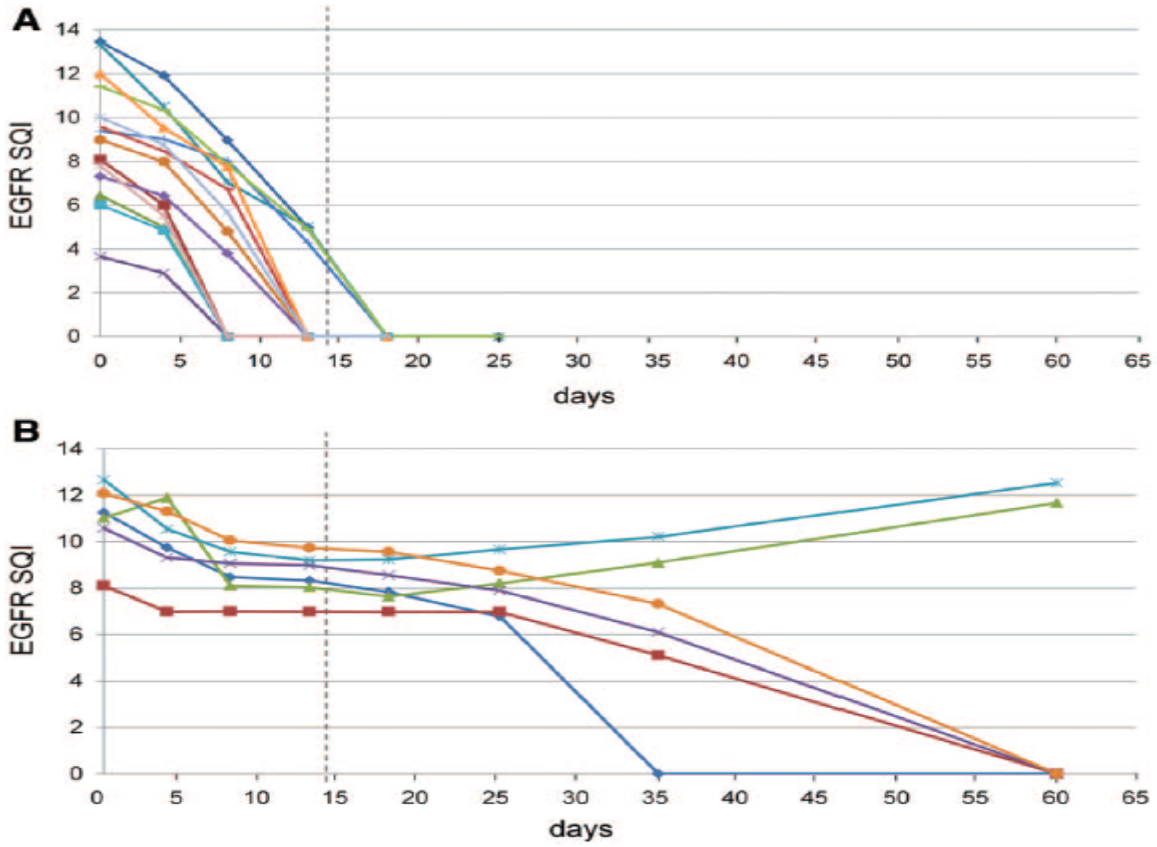
Treatment with AZD9291



Early Prediction of Response to Tyrosine Kinase Inhibitors by Quantification of *EGFR* Mutations in Plasma of NSCLC Patients

Antonio Marchetti, MD, PhD, John F. Palma, PhD,† Lara Felicioni, PhD,‡ Tommaso M. De Pas, MD,§ Rita Chiari, MD,‖ Maela Del Grammastro, PhD,* Giampaolo Filice, PhD,* Vienna Ludovini, PhD,‖ Alba A. Brandes, MD,¶ Antonio Chella, MD,# Francesco Malorgio, MD,** Flavio Guglielmi, MD,†† Michele De Tursi, MD,‡‡ Armando Santoro, MD,§§ Lucio Crinò, MD,‖‖ and Fiamma Buttitta, MD, PhD‡*

(*J Thorac Oncol.* 2015;10: 1437–1443)


A
EGFR SQI
14
12
10
8
6
4
2
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65
days
B
EGFR SQI
14
12
10
8
6
4
2
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65
days

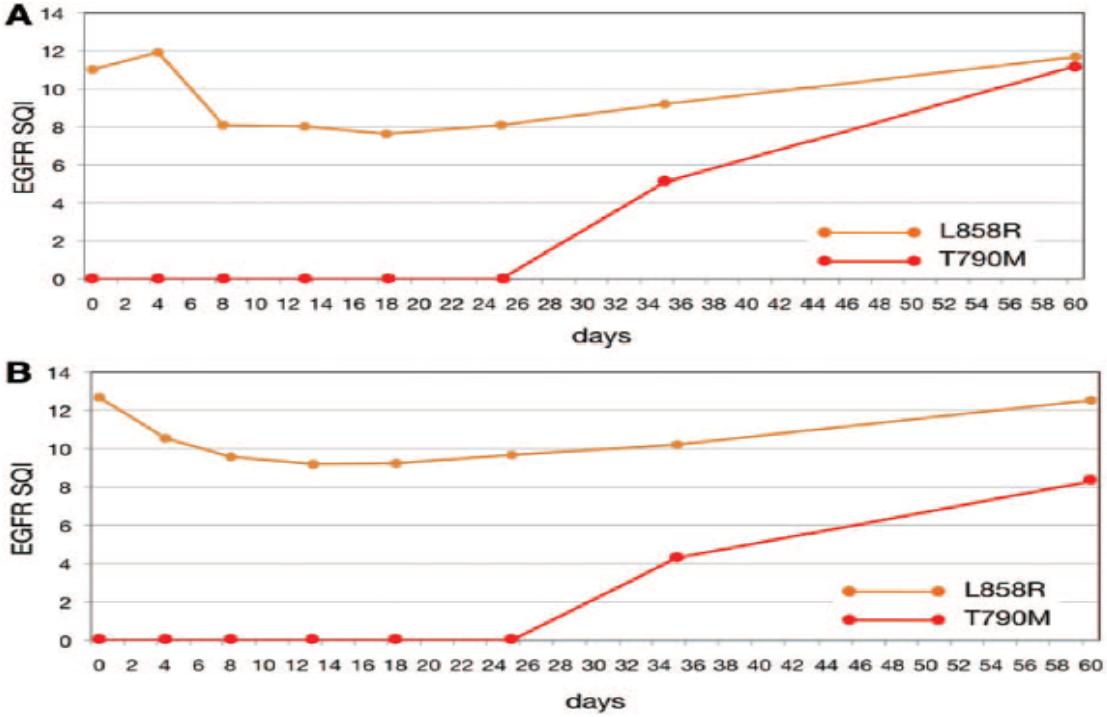


FIGURE 3. Quantification of mutated *EGFR* DNA from plasma of two slow responders with T790M mutation by the PCR test. The figures show the failure to clear the initial *EGFR* mutated DNA (L858R) and the emergence of T790M DNA during week 3 after initiating *EGFR* TKI treatment. PCR, polymerase chain reaction; SQI, semi-quantitative index; TKI, tyrosine kinase inhibitor.

an early increase in the circulating levels of the T790M mutation was observed. No T790M mutations were seen in serial plasma samples of the rapid responders. We therefore speculate that slow responders are more prone to develop early resistance. However, further clinical validation is required to assess the long-term impact of TKI treatment on rapid versus slow responders relative to progression-free and overall survival.

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

Annals of Oncology 29: 311–323, 2018
doi:10.1093/annonc/mdx766
Published online 5 December 2017

REVIEW

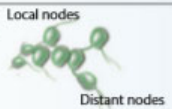
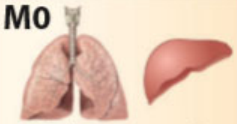
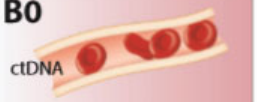
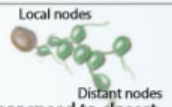
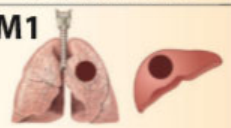
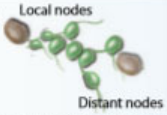
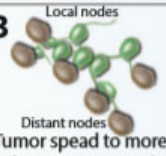
Incorporating blood-based liquid biopsy information into cancer staging: time for a TNMB system?

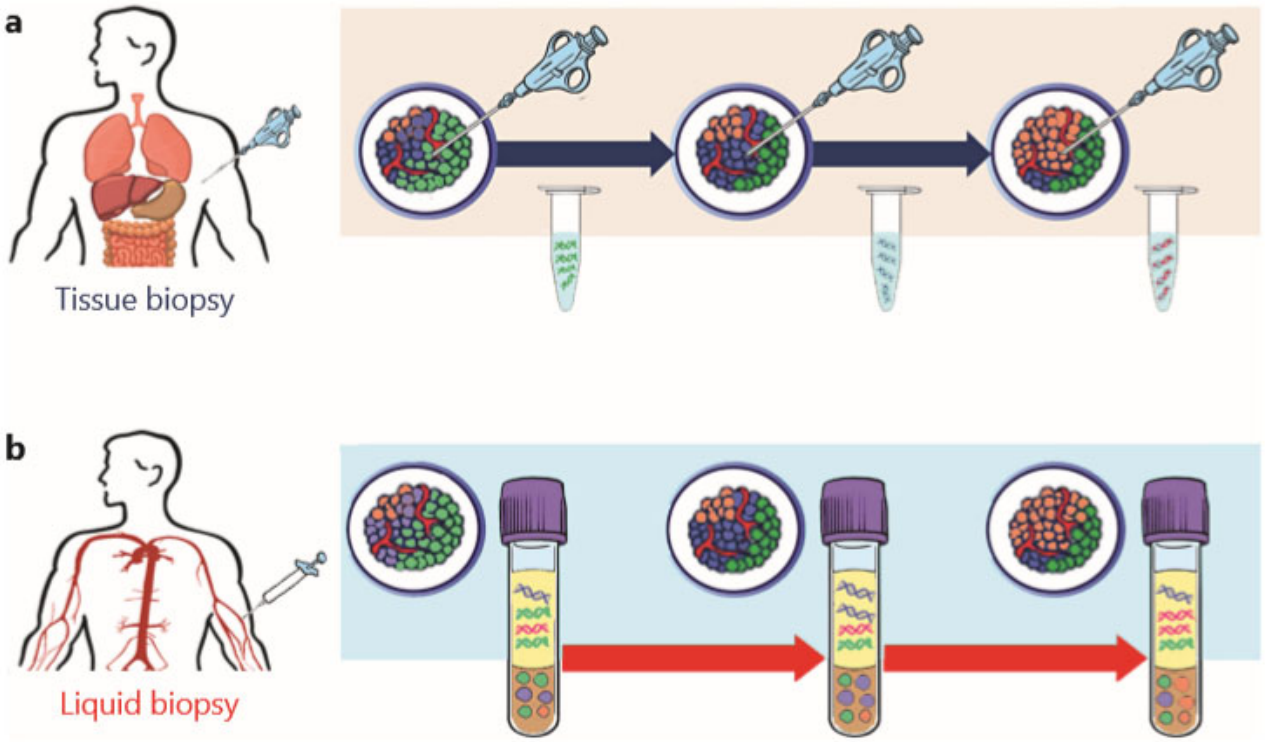
M. Yang[1,2,3,4], M. E. Forbes[1,2], R. L. Bitting[1,5], S. S. O'Neill[1,6], P.-C. Chou[1,2], U. Topaloglu[1,2], L. D. Miller[1,2], G. A. Hawkins[1,7], S. C. Grant[1,5], B. R. DeYoung[1,6], W. J. Petty[1,5], K. Chen[3,4*], B. C. Pasche[1,2,5] & W. Zhang[1,2*]

[1]Wake Forest Baptist Comprehensive Cancer Center, Wake Forest Baptist Medical Center, Winston-Salem; [2]Department of Cancer Biology, Wake Forest School of Medicine, Winston-Salem, USA; [3]Department of Epidemiology and Biostatistics, National Clinical Research Center for Cancer; [4]Key Laboratory of Cancer Prevention and Therapy, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China; [5]Section of Hematology and Oncology, Department of Internal Medicine; [6]Departments of Pathology; [7]Biochemistry, Wake Forest School of Medicine, Winston-Salem, USA

*Correspondence to: Prof. Wei Zhang, Cancer Genomics and Precision Oncology, Wake Forest Baptist Comprehensive Cancer Center, 1 Medical Center Blvd., Winston-Salem, NC 27157-1082, USA. Tel: +1-336-713-7508; E-mail: wezhang@wakehealth.edu

Prof. Kexin Chen, Department of Epidemiology and Biostatistics, Tianjin Medical University Cancer Institute and Hospital, Huanhu Xi Road, Tiyuan Bei, Hexi District, Tianjin 300060, China. Tel: +86-22-2337-2231; E-mail: chenkexin@tjmuch.com

T Tumor Size	N Lymph Node	M Metastasis	B Blood
T1  Tumor size/local invasion	**N0**  No regional lymph node invasion	**M0**  No distant metastasis	**B0**  No ctDNA mutations in blood
T2  Tumor size/local invasion	**N1**  Tumor spead to closest or small number of regional lymph nodes	**M1**  Distant metastasis	**B1**  ctDNA mutations in blood (can be further defined with more detailed quantification in the future)
T3  Tumor size/local invasion	**N2**  Tumor spead to an extent between N1 and N3		
T4  Tumor of any size that invades to other organs	**N3**  Tumor spead to more distant or regional numerous lymph nodes		



Pathobiology 2018;85:146–154
DOI: 10.1159/000473882

International Journal of
Molecular Sciences

MDPI

Review

Circulating Cell-Free DNA and Colorectal Cancer: A Systematic Review

Veronika Vymetalkova [1,2,3,*], Klara Cervena [1,2], Linda Bartu [1,2] and Pavel Vodicka [1,2,3]

1 Institute of Experimental Medicine of the Czech Academy of Sciences, Videnska 1083, 142 00 Prague, Czech Republic; klara.cervena13@seznam.cz (K.C.); lindabartu@gmail.com (L.B.); pavel.vodicka@iem.cas.cz (P.V.)
2 Institute of Biology and Medical Genetics, 1st Medical Faculty, Charles University, Albertov 4, 128 00 Prague, Czech Republic
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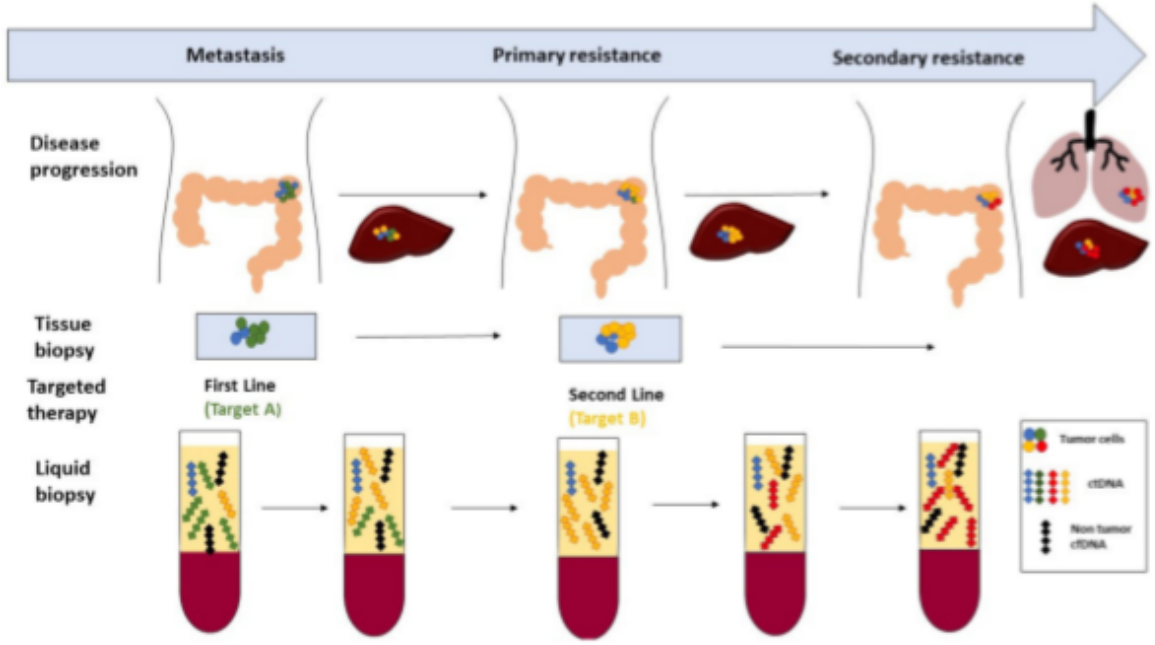
* Correspondence: veronika.vymetalkova@iem.cas.cz

Received: 19 September 2018; Accepted: 24 October 2018; Published: 26 October 2018

2.2. Cell-Free DNA and Colorectal Cancer

Analysis of ctDNA is a promising new tool in oncology. ctDNA mutational content can provide invaluable information on the genetic background of a tumor, and assist oncologist in deciding on therapy, or in following the residual disease (Figure 2, Table 3).



Thank You!!!