GENE EXPRESSION IN DEVELOPMENT AND DISEASE COURSE, UCT

DRUGS THAT MODULATE TRANSCRIPTION FACTORS

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OUTLINE

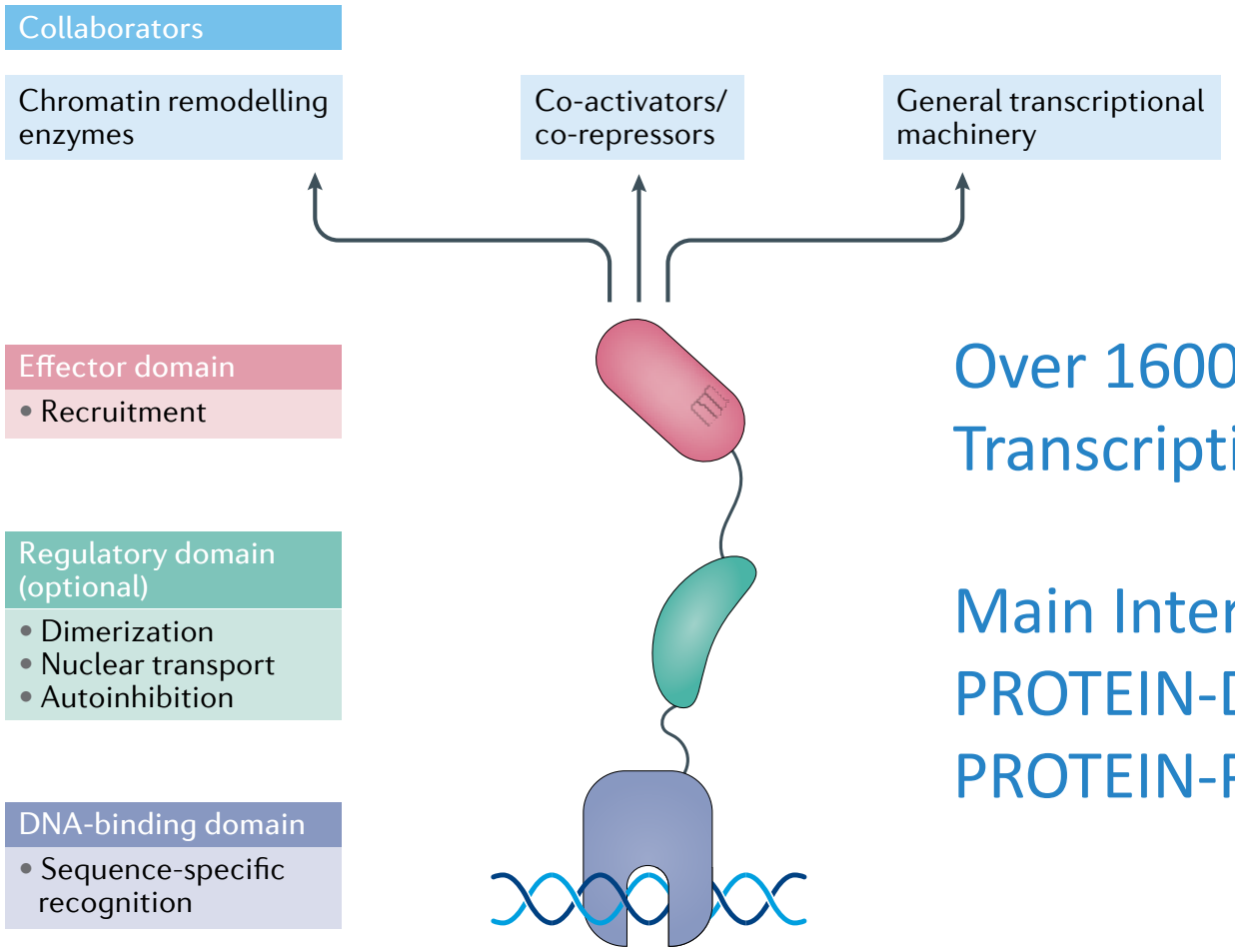
- General aspects
- Transcription factors in Cancer
- Targeting nuclear hormone receptors
- From undruggable targets to reality
  - Multiple possibilities of alteration of the TF function
  - Trabectedin as a successful example

FIRST THINGS FIRST

- Definition…
  - Transcription factors (TF) are proteins involved in the process of converting, or transcribing, DNA into RNA (https://www.nature.com/scitable/definition/transcription-factor-transcription-factors-167/)
  - Transcription factors (TFs) recognize specific DNA sequences to control chromatin and transcription, forming a complex system that guides expression of the genome (Lambert et al., 2018. Cell 172: 650)

Key features:
DNA Binding
Transcription Regulation

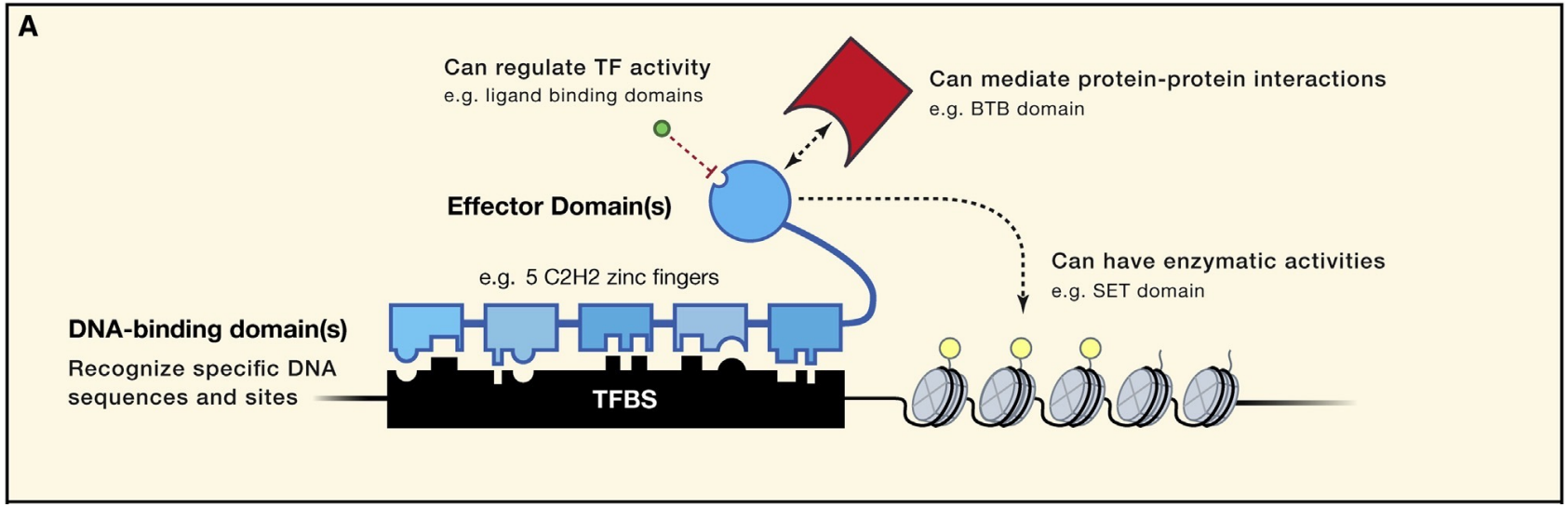
THE ANATOMY OF A TRANSCRIPTION FACTOR



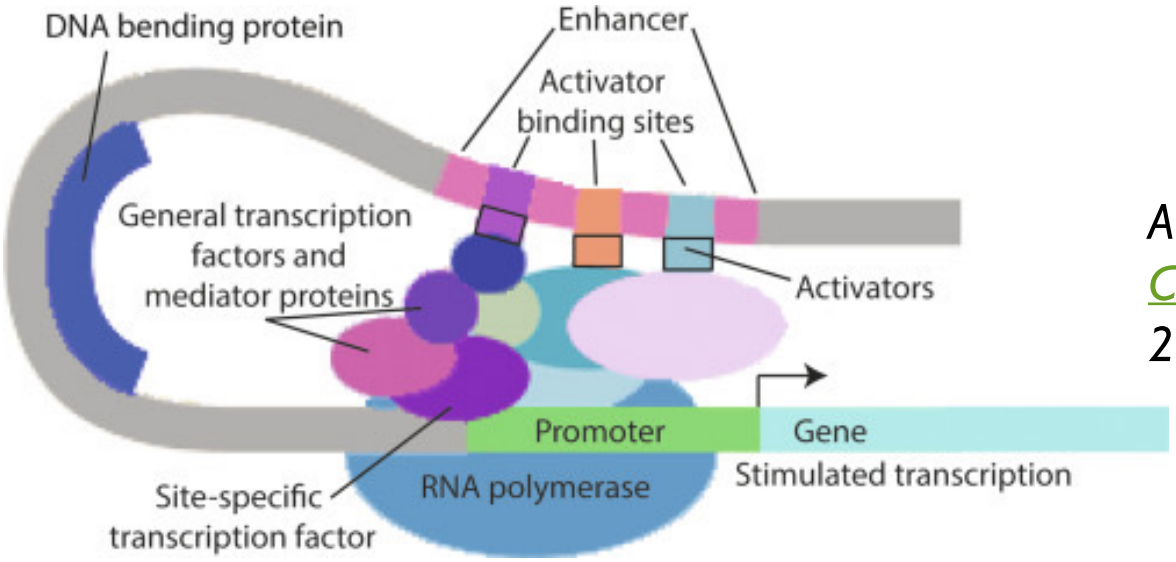
Over 1600 described Transcription Factors!

Main Interactions:
PROTEIN-DNA
PROTEIN-PROTEIN

Henley & Koehler, 2021. Nat. Rev. Drug Discovery, vol 20, pag 669.



(Lambert et al., 2018. Cell 172: 650)



Arie S. Mobley, in Neural Stem Cells and Adult Neurogenesis, 2019

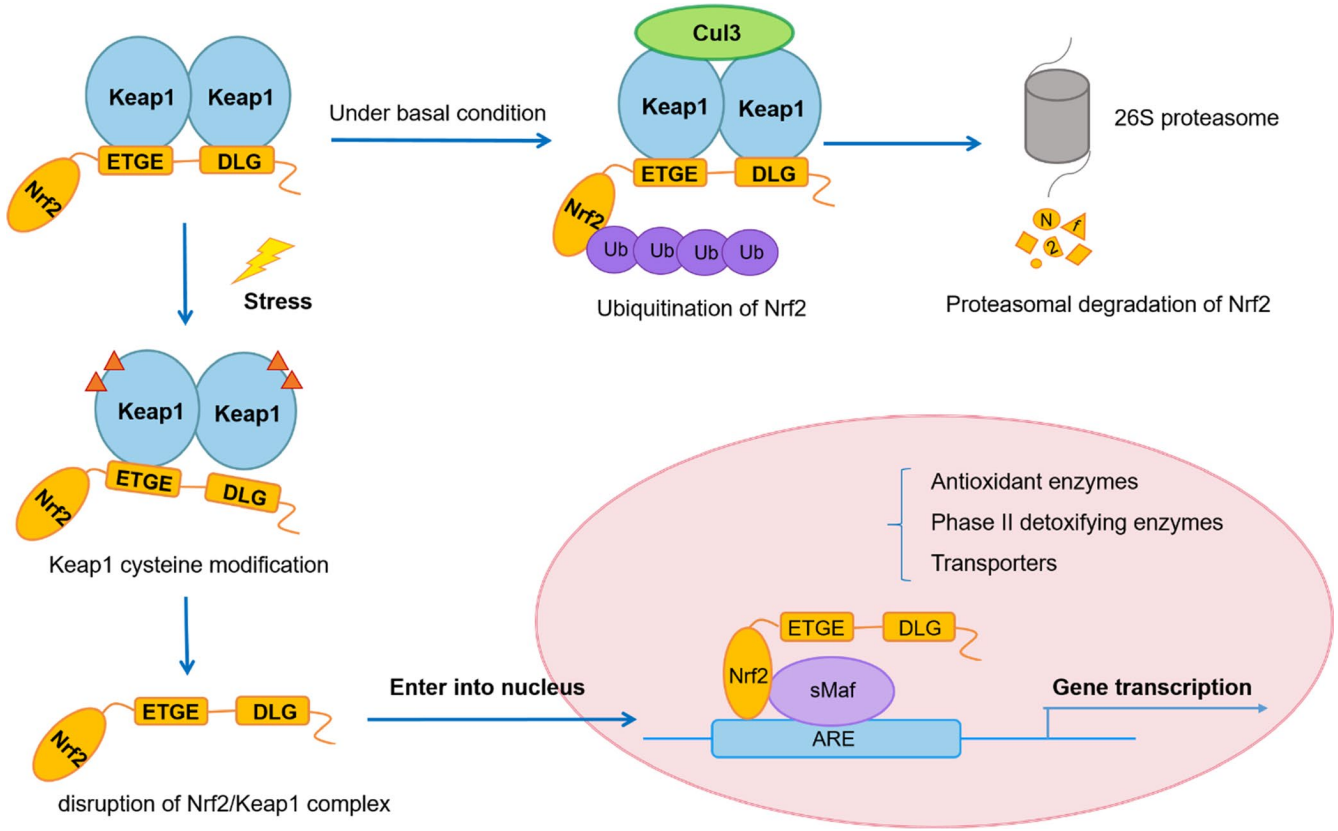


FIGURE 1 Nrf2/Keap1 signaling pathway. Under basal conditions, Nrf2 binds to Keap1 by its two motifs (ETGE and DLG) and activates Cul3-mediated ubiquitination followed by proteasomal degradation. Under stress conditions, due to the modification of Keap1 cysteine residues, Nrf2 dissociates from Keap1 and translocates into the nucleus. Nrf2 then forms a heterodimer with sMaf protein and binds to ARE to initiate the transcription of various downstream genes

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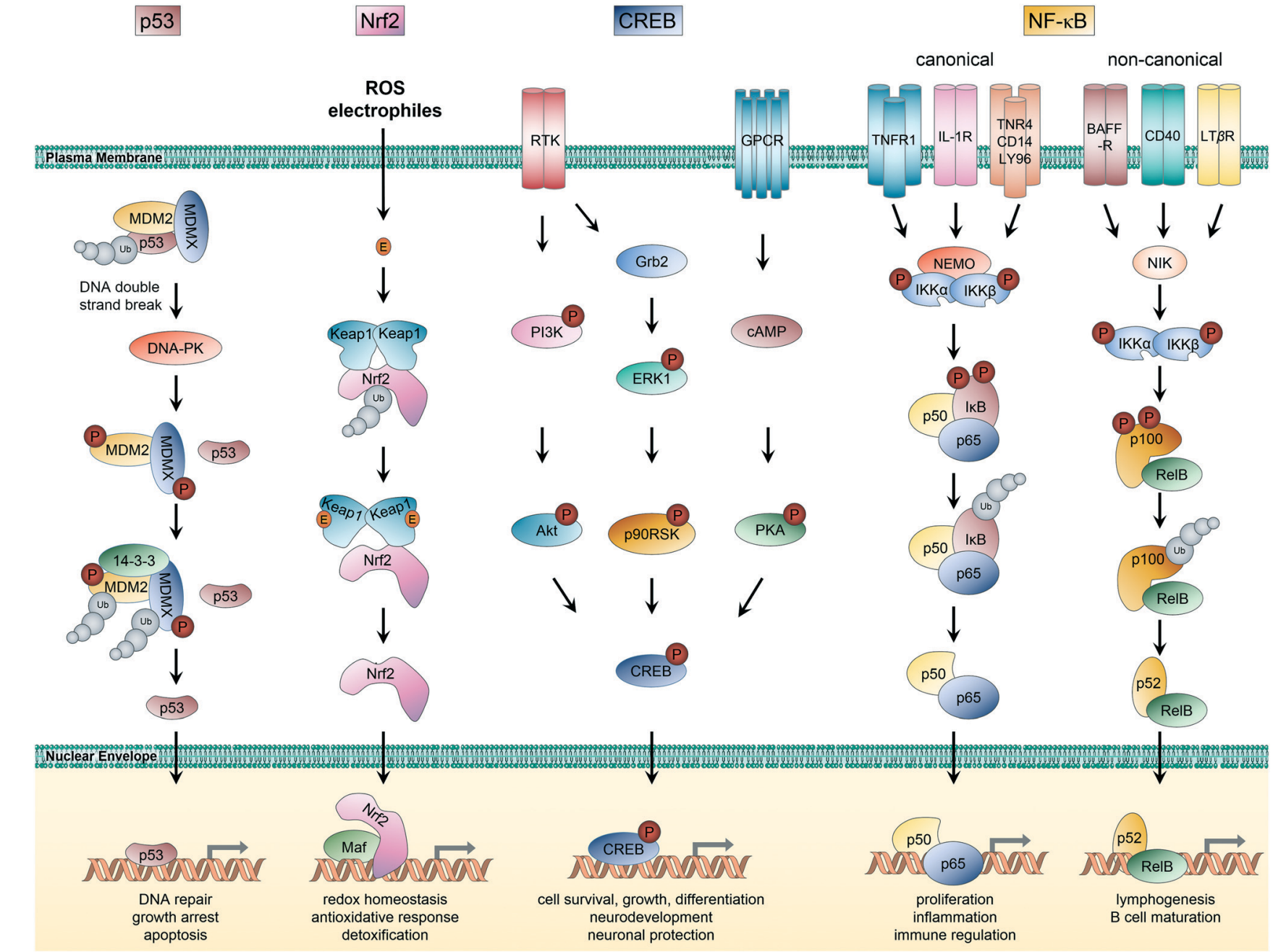
DOI: 10.1002/cam4.2101

REVIEW

WILEY Cancer Medicine Open Access

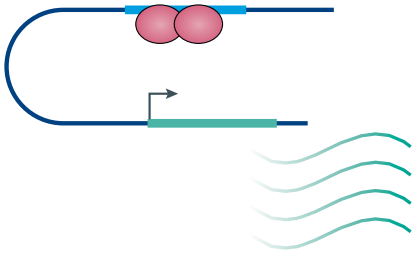
Nrf2 in cancers: A double-edged sword

Shijia Wu[1] | **Hong Lu**[2] | **Yongheng Bai**[1]

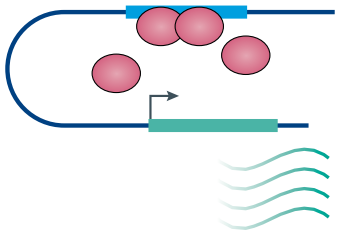


DOI: 10.1039/c8md00273h

TF IN DISEASE



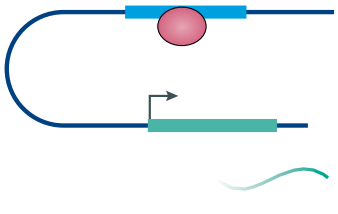
Normal transcription



↑Transcription

Upregulation

- Amplification
- Gain of function
- Pathway overactivation

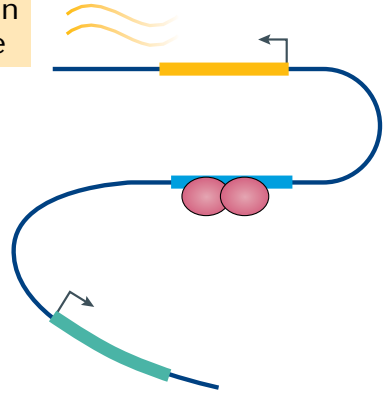


↓Transcription

Downregulation

- Loss of function
- Overactivation of repressors

Transcription of new gene

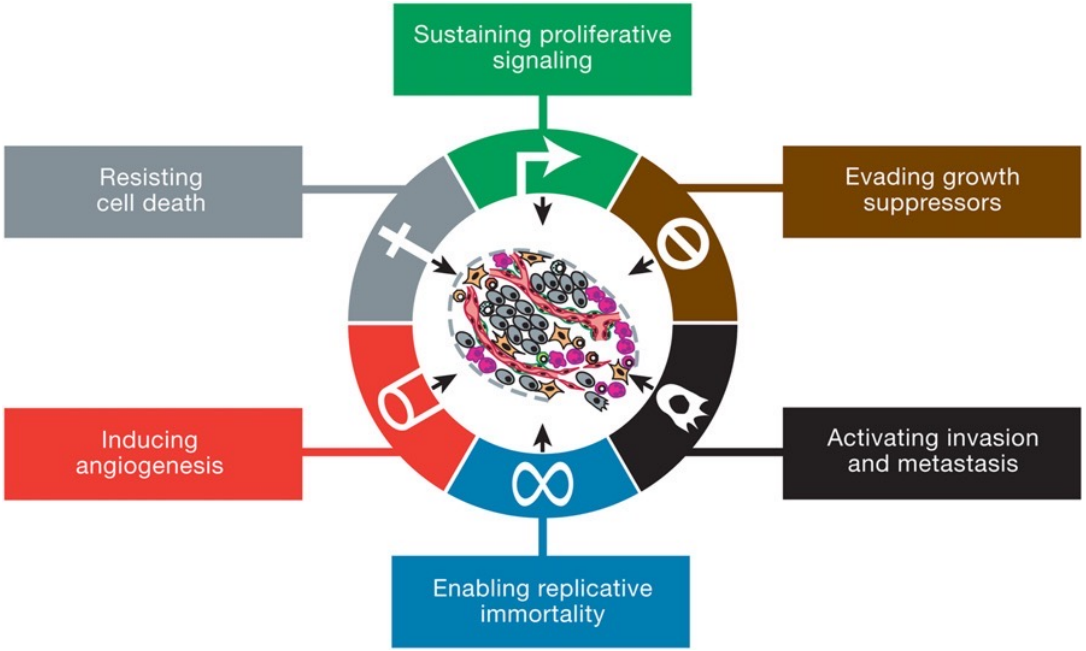


Changes in target genes

- Chromatin architecture shifts
- Gene translocations
- Fusion transcription factors

Henley & Koehler, 2021. Nat. Rev. Drug Discovery, vol 20, pag 669.

TRANSCRIPTION FACTORS IN CANCER



- Transcription factors account for 20% of oncogenes in cancer

Hanahan and Weinberg, 2000. Cell 100: 57.
Hanahan and Weinberg, 2011. Cell 144: 646.

Table 1. List of the 294 known or candidate oncogenic transcription factors and regulators [1].

ABL1	CEBPA	ERCC3	HIST1H2BE	MDM4	PAX7	SMARCA4	TFPT
AFF1	CHD1	ERCC6	HIST1H2BG	MED12	PAX8	SMARCB1	THRAP3
AFF3	CHD2	ERF	HLF	MEF2B	PBX1	SMARCD1	TLX1
AFF4	CHD4	ERG	HMGA1	MEF2C	PEG3	SMARCE1	TLX3
APC	CHD5	ESPL1	HMGA2	MEN1	PER1	SMURF2	TNFAIP3
AR	CHD7	ESR1	HOXA11	MITF	PHF3	SOX2	TP53
ARID1A	CIC	ETS1	HOXA13	MKL1	PHF6	SOX5	TRIM24
ARID1B	CIITA	ETV1	HOXA7	MLLT1	PHOX2B	SOX9	TRIM33
ARID3B	CNOT3	ETV4	HOXA9	MLLT10	PLAG1	SRCAP	TRIP11
ARID5B	CREB1	ETV5	HOXC11	MLLT3	PML	SS18L1	TRPS1
ARNT	CREB3L1	ETV6	HOXC13	MLLT6	PMS1	SSB	TRRAP
ARNT2	CREBBP	EWSR1	HOXD11	MYB	PNN	SSX1	TSC22D1
ASB15	CRTC1	EYA4	HOXD13	MYBL1	POU2AF1	SSX2	TSHZ3
ASXL1	CSDE1	EZH2	ID3	MYC	POU2F2	SSX4	VHL
ATF1	CTCF	FEV	IRF2	MYCN	POU5F1	STAT3	WHSC1
ATF7IP	CTNNB1	FLI1	IRF4	MYOD1	PPARG	STAT4	WHSC1L1
ATM	DACH1	FOXA1	IRF6	NCOA1	PRDM1	STAT5B	WT1
ATRX	DACH2	FOXE1	IRF8	NCOA2	PRDM16	STAT6	WWP1
BAZ2B	DAXX	FOXL2	IRX6	NCOA4	PRDM9	SUFU	WWTR1
BCL11A	DDB2	FOXP1	JUN	NCOR1	PRRX1	SUZ12	XBP1
BCL11B	DDIT3	FOXQ1	KHDRBS2	NCOR2	PSIP1	TAF1	XPC
BCL3	DDX5	FUBP1	KHSRP	NEUROG2	RARA	TAF15	ZBTB16
BCL6	DEK	FUS	KLF2	NFE2L2	RB1	TAL1	ZBTB20
BCLAF1	DIP2C	FXR1	KLF4	NFE2L3	RBM15	TAL2	ZFP36L1
BCOR	DNMT1	GATA1	KLF5	NFIB	RBMX	TBX18	ZFX
BRCA1	DNMT3A	GATA2	KLF6	NFKB2	REL	TBX22	ZHX2
BRCA2	DOT1L	GATA3	LDB1	NFKBIA	RUNX1	TBX3	ZIC3
BRD7	EED	GLI3	LMO1	NONO	RUNX1T1	TCEA1	ZIM2
BRD8	EGR2	GTF2I	LMO2	NOTCH2	RXRA	TCEB1	ZNF208
BRIP1	ELAVL2	HDAC9	LMX1A	NOTCH3	SALL3	TCERG1	ZNF226
BRPF3	ELF3	HEY1	LYL1	NPM1	SATB2	TCF12	ZNF331
BTG1	ELF4	HIST1H1B	LZTR1	NR3C2	SETBP1	TCF3	ZNF384
BTG2	ELK4	HIST1H1C	MAF	NR4A3	SFPQ	TCF7L2	ZNF469
CBFA2T3	ELL	HIST1H1D	MAFA	NSD1	SIN3A	TFAP2D	ZNF595
CBFB	EP300	HIST1H1E	MAFB	OLIG2	SMAD2	TFDP1	ZNF638
CDX2	EPC1	HIST1H2BC	MAML1	PAX3	SMAD4	TFE3	
CDX4	ERCC2	HIST1H2BD	MAX	PAX5	SMARCA1	TFEB	

[1] This list is obtained by crossing data from the known and candidate cancer genes lists (http://ncg.kcl.ac.uk/statistics.php) with the list of known human transcription factors [5]).

TRANSCRIPTION FACTORS IN CANCER

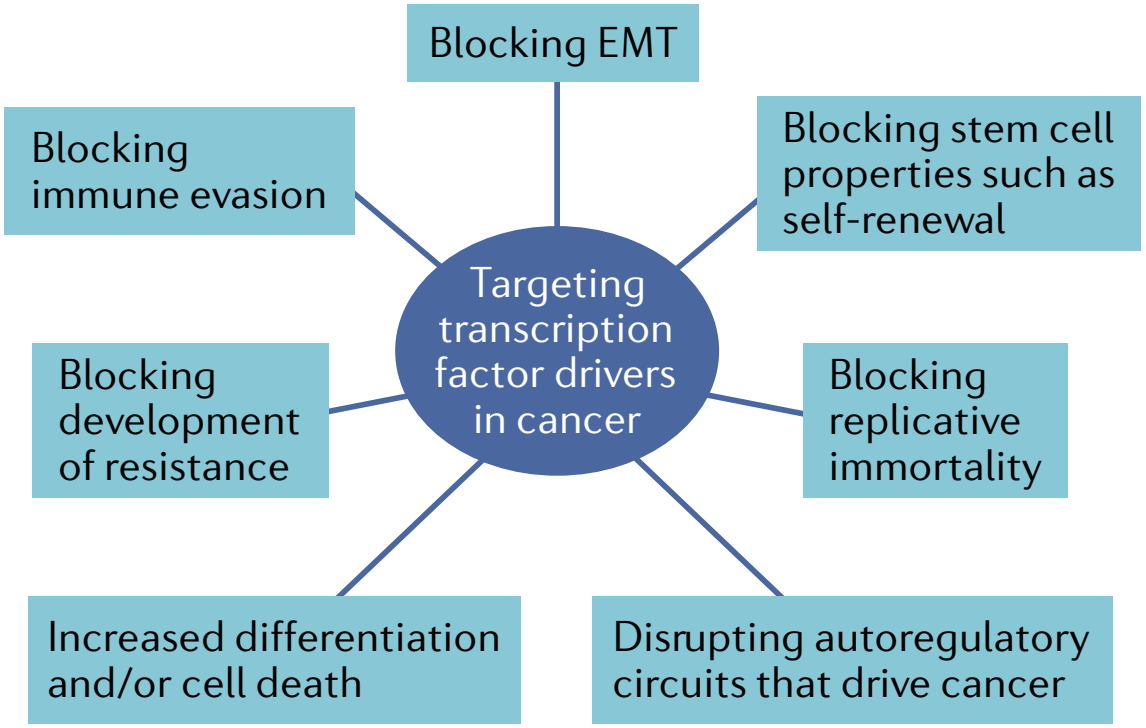


Fig. 1 | **Targeting transcription factor drivers in cancer.** Schematic showing possible beneficial outcomes of inhibiting the activity of transcription factor drivers in cancer. EMT, epithelial-to-mesenchymal transition.

Bushweller. *Nature Reviews in Cancer* **2019**, *19*; 611.

SOME EXAMPLES:

Table 1 | **Selected examples of TFs that drive disease**

TF	Associated diseases	Dysregulation mechanisms	Refs
Cancer			
MYC	Various forms of cancer	Amplifies oncogenic transcriptional programmes	89,90
MYB	Various forms of cancer	Overactivation by gene duplication, overexpression and genetic fusions to other proteins	84
E2F	Various forms of cancer	Overactivation by dysregulation of co-repressor pRB	19,287
TAL1	T cell acute lymphoblastic leukaemia	Overexpression and overactivation	288
PAX3-FOXO1	Alveolar rhabdomyosarcoma	Oncogenic fusion TF, dysregulates muscle development transcriptional programmes	95,289
p53	Various forms of cancer	Downregulation by the ubiquitin–proteasome system or loss-of-function mutations	141,142

Henley & Koehler, 2021. Nat. Rev. Drug Discovery, vol 20, pag 669.

Biochemical Pharmacology 107 (2016) 1–13

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Research update

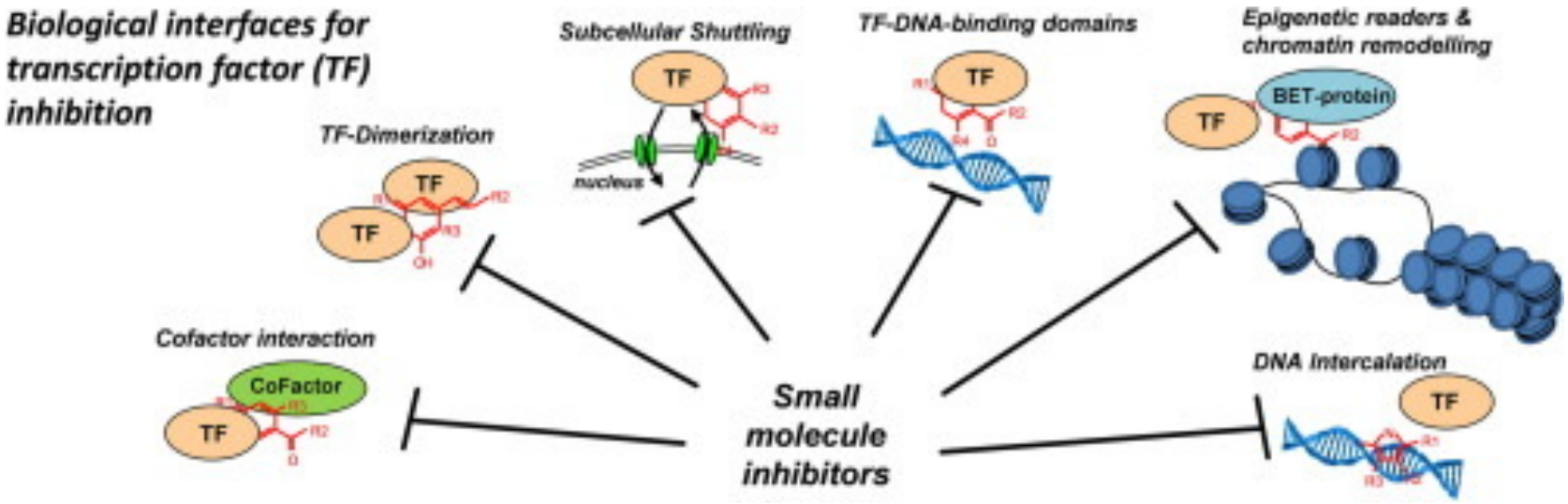
Targeting transcription factors by small compounds—Current strategies and future implications

Judith Hagenbuchner[b], Michael J. Ausserlechner[a,*]

[a] Department of Pediatrics I, Medical University Innsbruck, Innrain 66, A-6020 Innsbruck, Austria
[b] Department of Pediatrics II, Medical University Innsbruck, Innrain 66, A-6020 Innsbruck, Austria

- Ligand-activated nuclear receptors – ligand binding pocket – “easy” to modulate by small molecules.
- Non-ligand transcription factors – huge interacting surface between transcription factor and DNA and subject to significant changes during DNA-binding – UNDRUGGABLE

HOW TO MODULATE A TF:



J. Hagenbuchner, M.J. Ausserlechner. Biochemical Pharmacology *107* (2016) 1–713

TARGETING TRANSCRIPTION FACTORS:

Their inhibition (or activation) at the expression level

Their inhibition through physical degradation

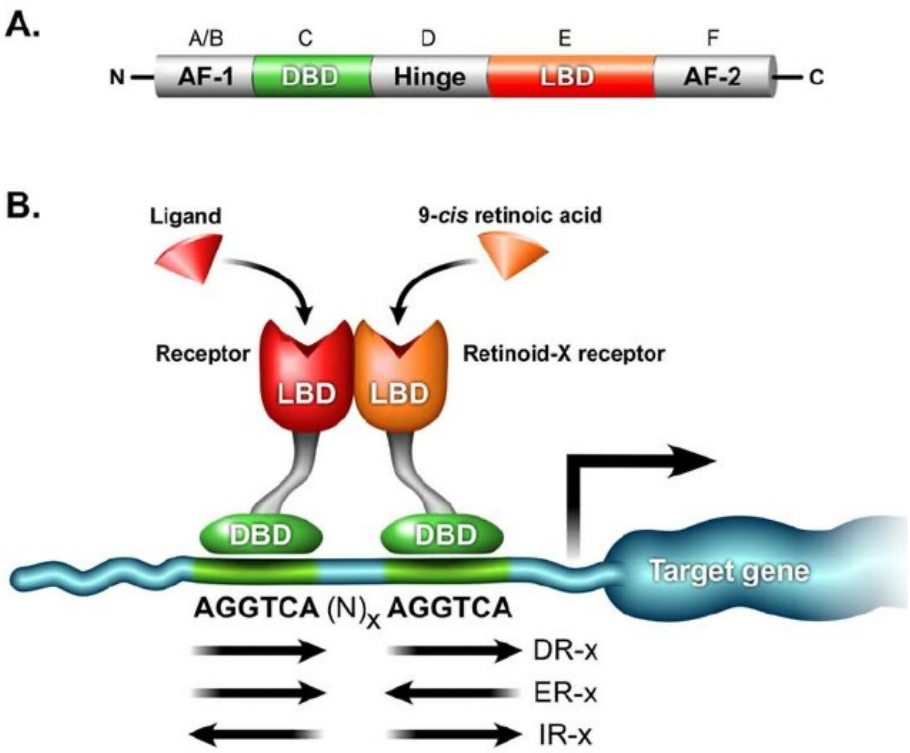
Their inhibition (or activation) at the protein/protein interaction level,

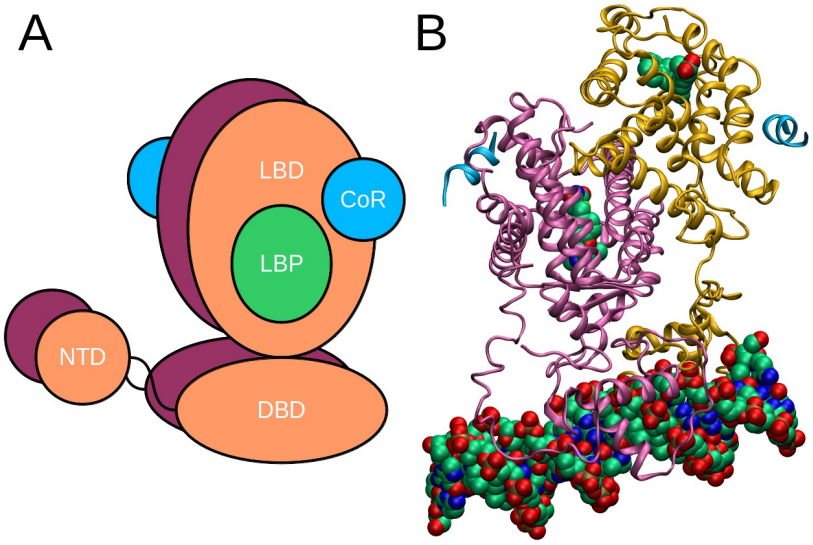
Their inhibition (or activation) through the binding of a ligand-based molecule in an activation/inhibition pocket

Their inhibition (or activation) at the protein/DNA binding level

Lambert et al., 2018. Molecules, 23, 1479; doi:10.3390/molecules23061479

NUCLEAR RECEPTORS AS TARGETS



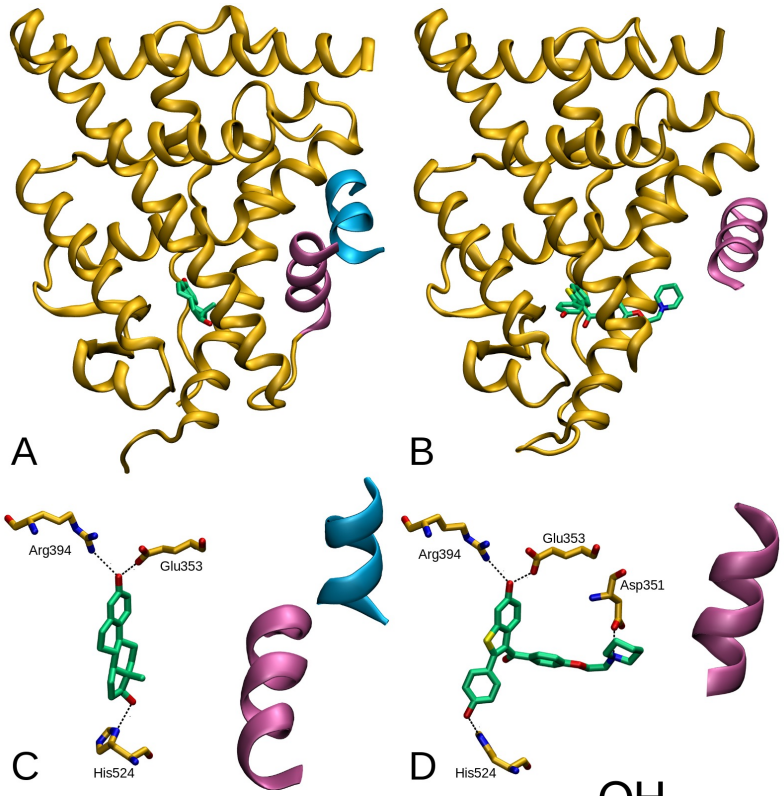


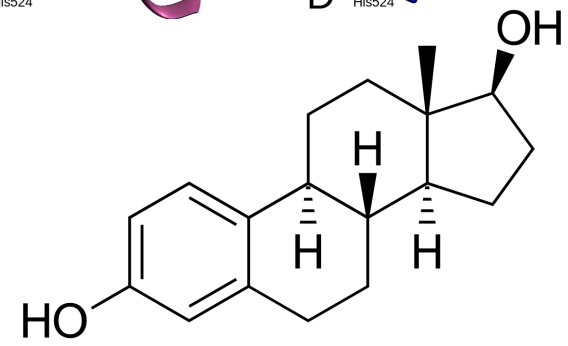
Kronenberger et al., DOI: 10.5772/59666

Ligand Binding Pocket – "High Affinity" binding site – Good for drugs
X-ray crystal structure (rosiglitazone - PPARγ)

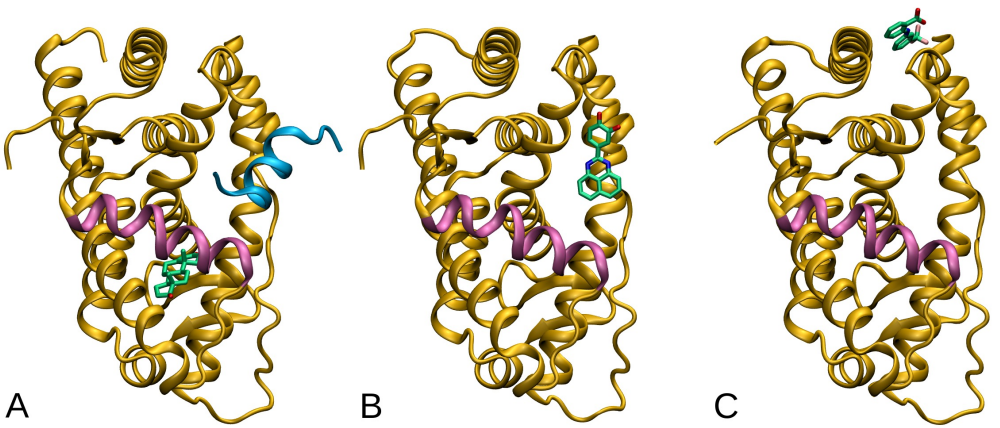
EXAMPLES

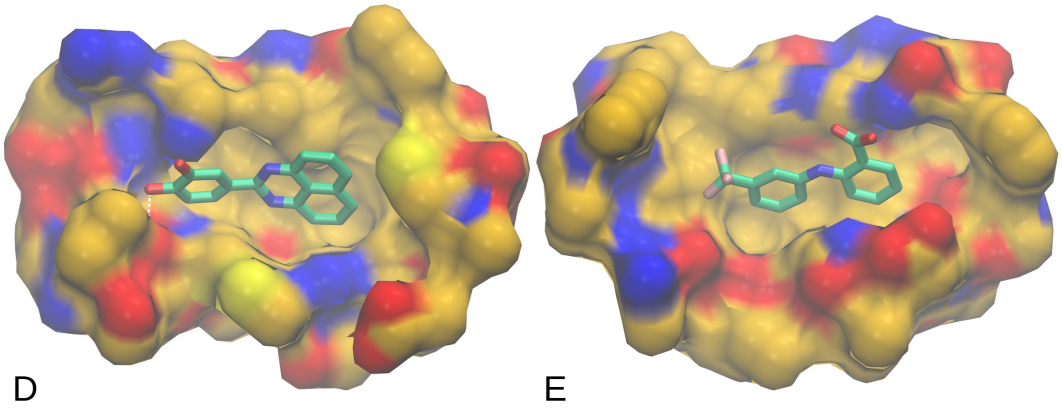
Estrogen Receptor

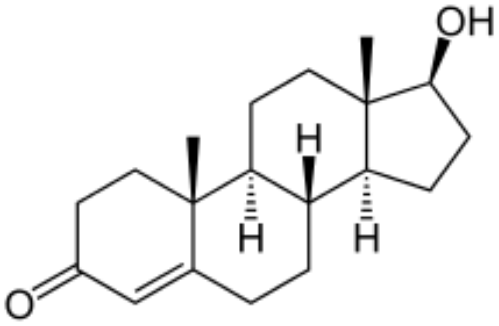




Androgen Receptor

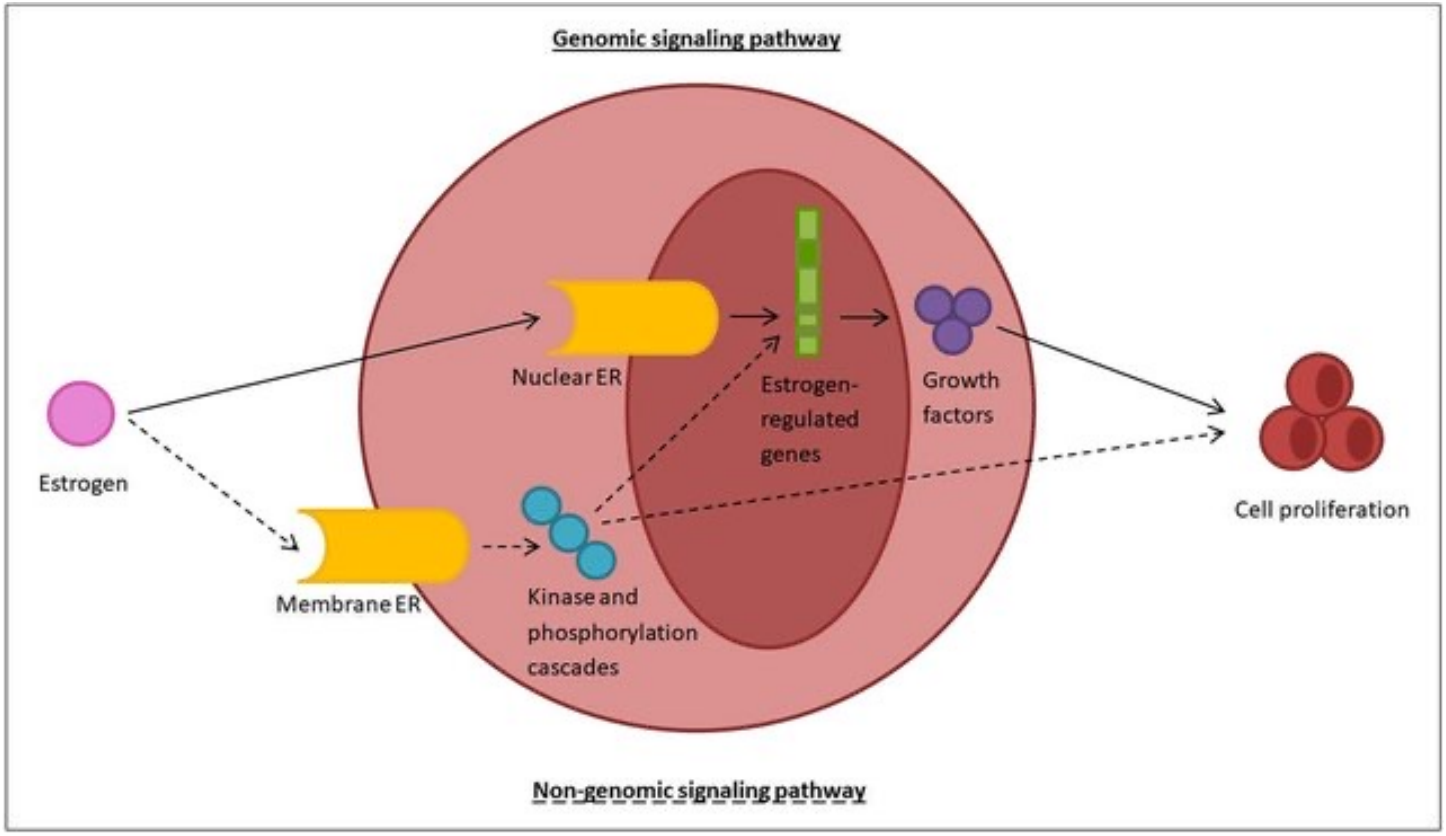






Kronenberger et al.,
DOI: 10.5772/59666

TROPHIC FUNCTIONS OF REPRODUCTIVE HORMONES



Breast Cancer Research and Treatment (2019) 175:17–25
https://doi.org/10.1007/s10549-019-05154-7

REVIEW

Check for updates

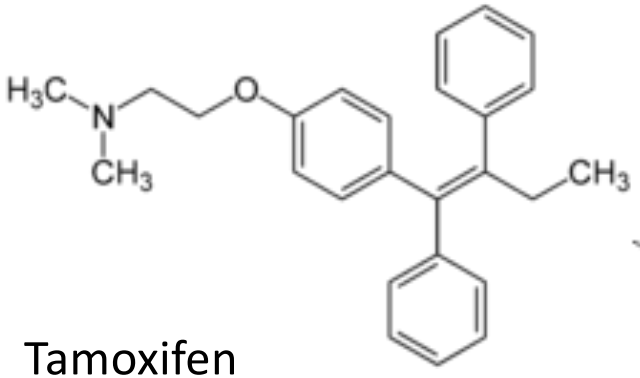
Tamoxifen and pregnancy: an absolute contraindication?

T. N. Schuurman[1] · P. O. Witteveen[2] · E. van der Wall[2] · J. L. M. Passier[3] · A. D. R. Huitema[4,5] · F. Amant[1,6,7] · C. A. R. Lok[1]

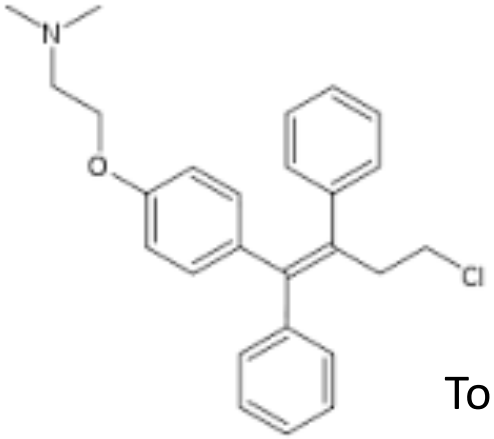
Received: 22 January 2019 / Accepted: 28 January 2019 / Published online: 1 February 2019

ANTI-ESTROGENIC DRUGS

Targeting the ER



Tamoxifen



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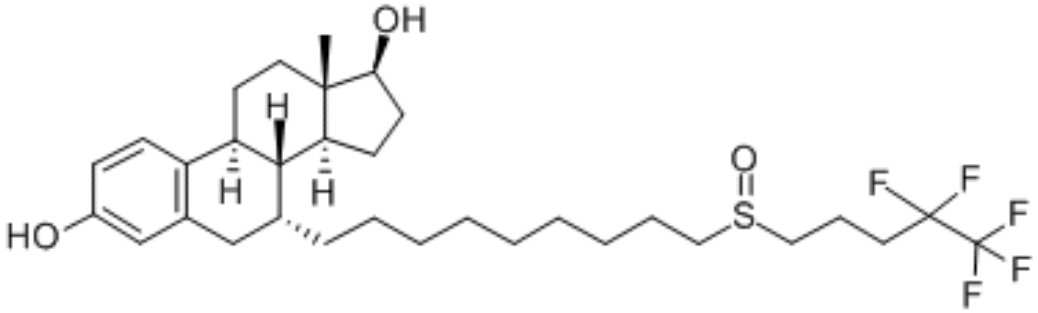


Fulvestrant

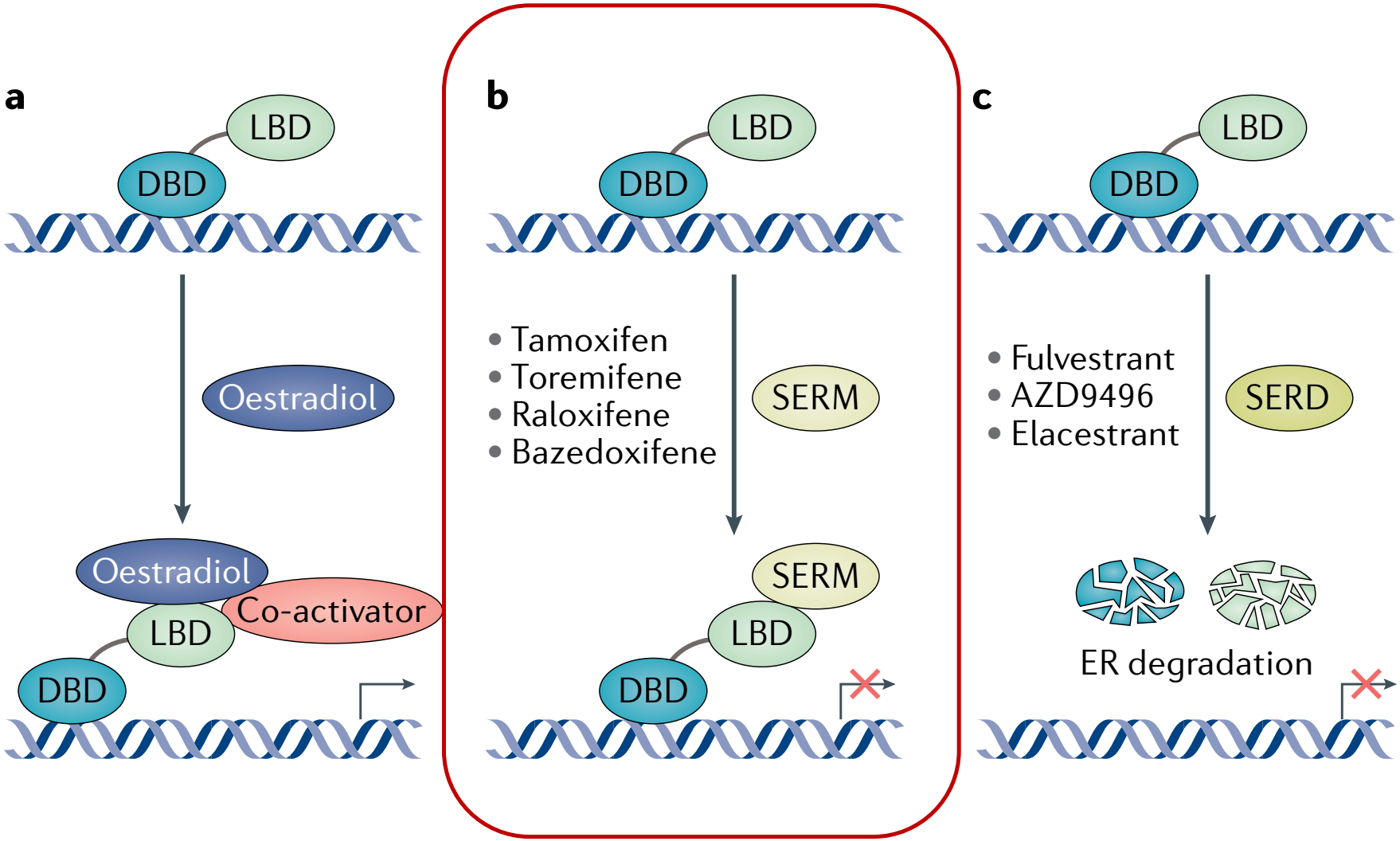
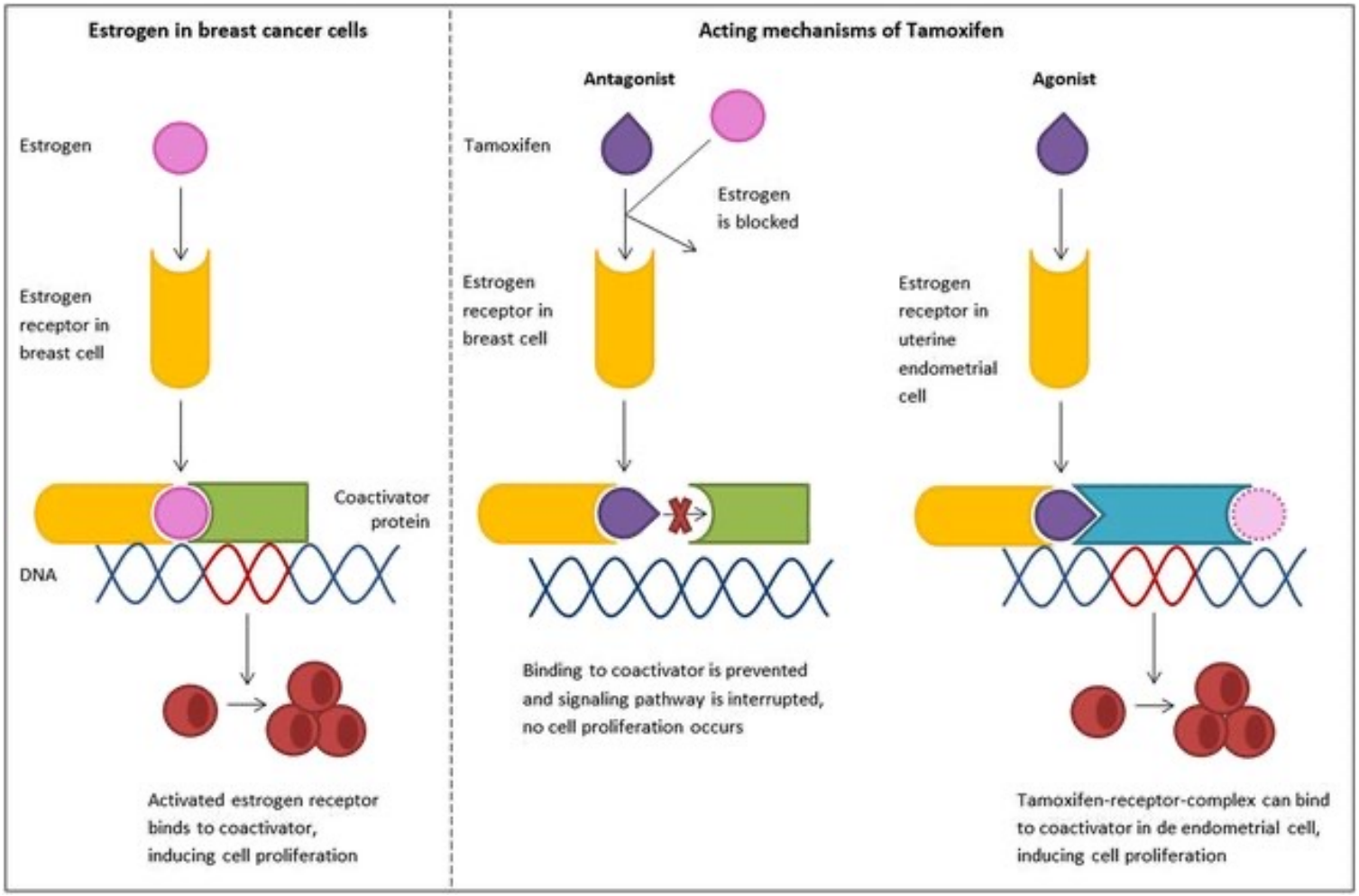


Fig. 2 | **Targeting oestrogen receptor function.**

Bushweller. *Nature Reviews in Cancer* **2019**, *19*; 611.



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REVIEW

Tamoxifen and pregnancy: an absolute contraindication?

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Received: 22 January 2019 / Accepted: 28 January 2019 / Published online: 1 February 2019

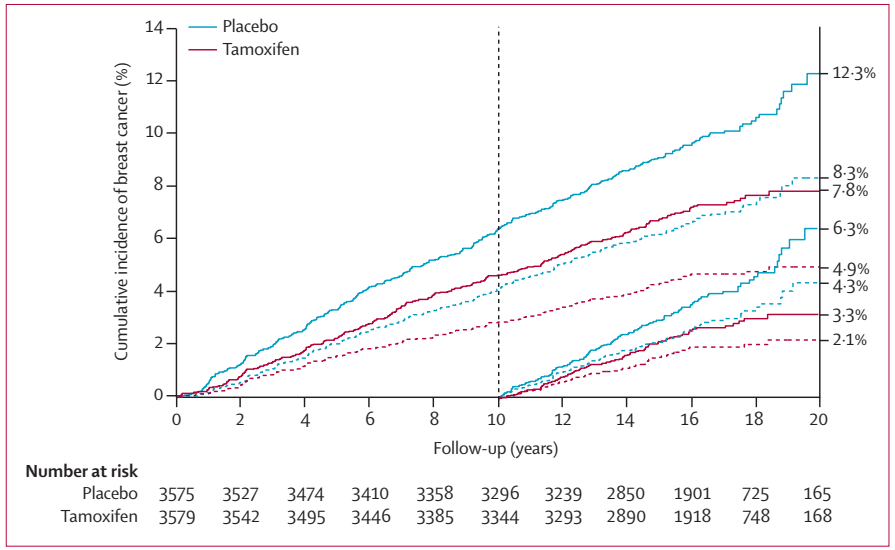


Figure 1: **Cumulative incidence of breast cancers over time**
All breast cancers (solid lines) and invasive oestrogen receptor-positive breast cancers (dashed lines), according to treatment group and duration of follow-up.

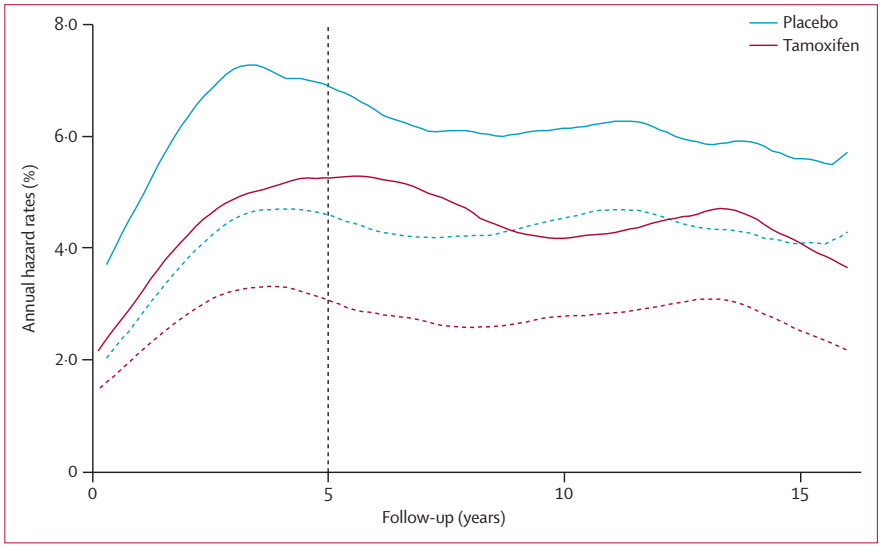


Figure 2: **Smoothed annual hazard rate curves for breast cancer**
All breast cancers (solid lines) and invasive oestrogen receptor-positive breast cancers (dashed lines), according to treatment group.

Tamoxifen for prevention of breast cancer: extended long-term follow-up of the IBIS-I breast cancer prevention trial

*Jack Cuzick, Ivana Sestak, Simon Cawthorn, Hisham Hamed, Kaija Holli, Anthony Howell, John F Forbes, on behalf of the IBIS-I Investigators**

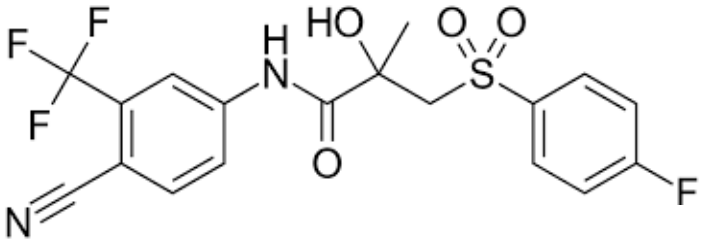
Summary

Background **Four previously published randomised clinical trials have shown that tamoxifen can reduce the risk of breast cancer in healthy women at increased risk of breast cancer in the first 10 years of follow-up. We report the long-term follow-up of the IBIS-I trial, in which the participants and investigators remain largely masked to treatment allocation.**

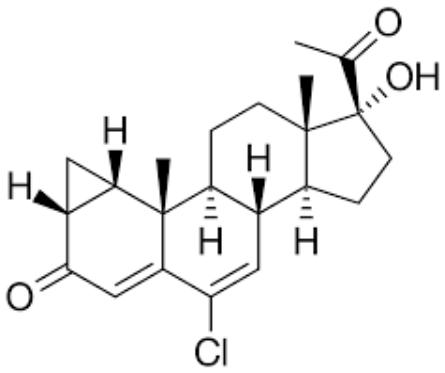
Lancet Oncol 2015; 16: 67–75
Published Online
December 11, 2014

ANTI-ANDROGENIC DRUGS

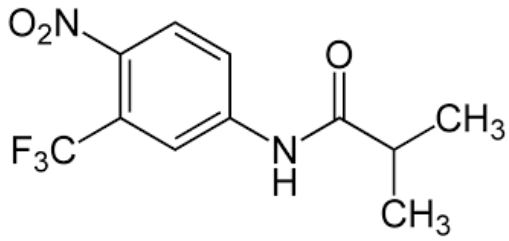
TARGETING THE AR



Bicalutamide



Cyproterone

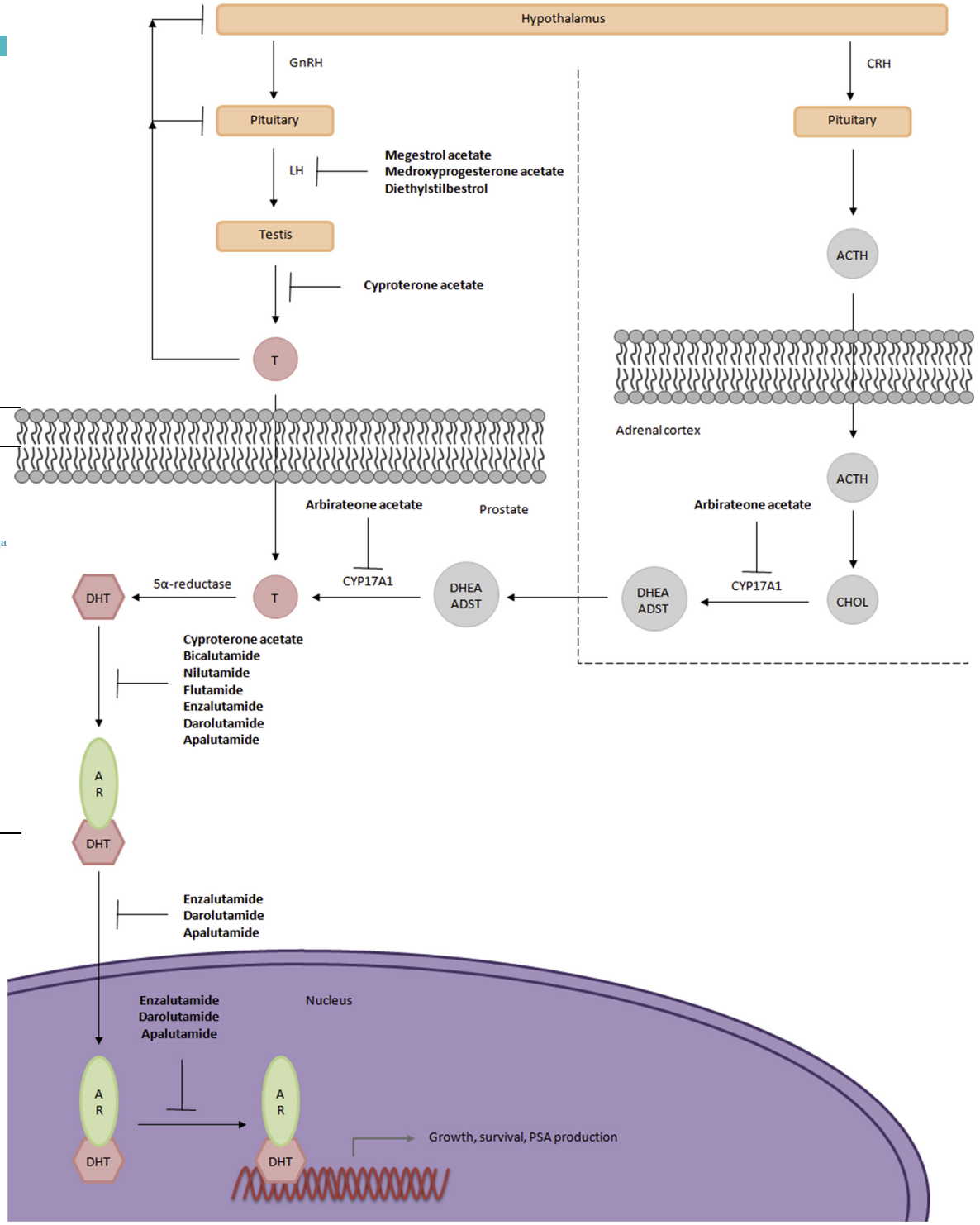


Flutamide

ANTI-ANDROGENIC DRUGS

Table 1
Applications of selected anti-androgens.

Drug	Application
Cyproterone acetate	Prostate cancer[a] Hirsutism (excessive hair loss)[a] Alopecia (hair loss)[a] Early puberty[a] Amenorrhoea (lack of menstrual period)[a] Acne[a] Transgender therapy[b]
Flutamide	Prostate cancer[a] Acne and seborrhoea[b] Hirsutism[b] Alopecia (hair loss)[b] Hidradenitis suppurativa[b]
Bicalutamide	Prostate cancer[a] Breast cancer[b]
Enzalutamide	Prostate cancer[a] Breast cancer[b] Ovarian cancer[b] Kidney cancer (before surgery)[b]



European Journal of Pharmacology 866 (2020) 172783
Contents lists available at ScienceDirect
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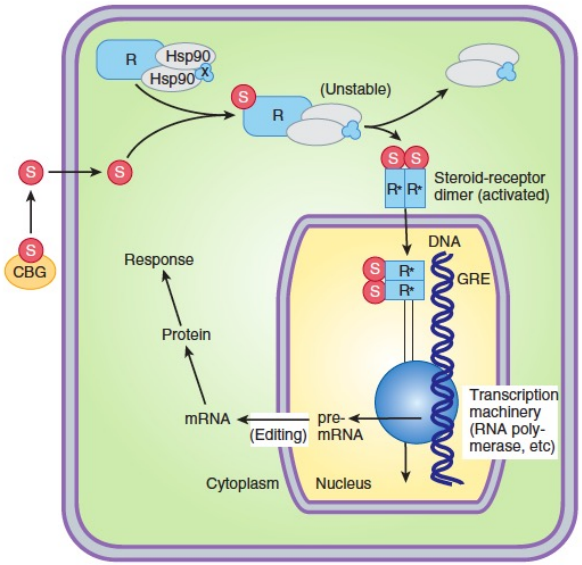
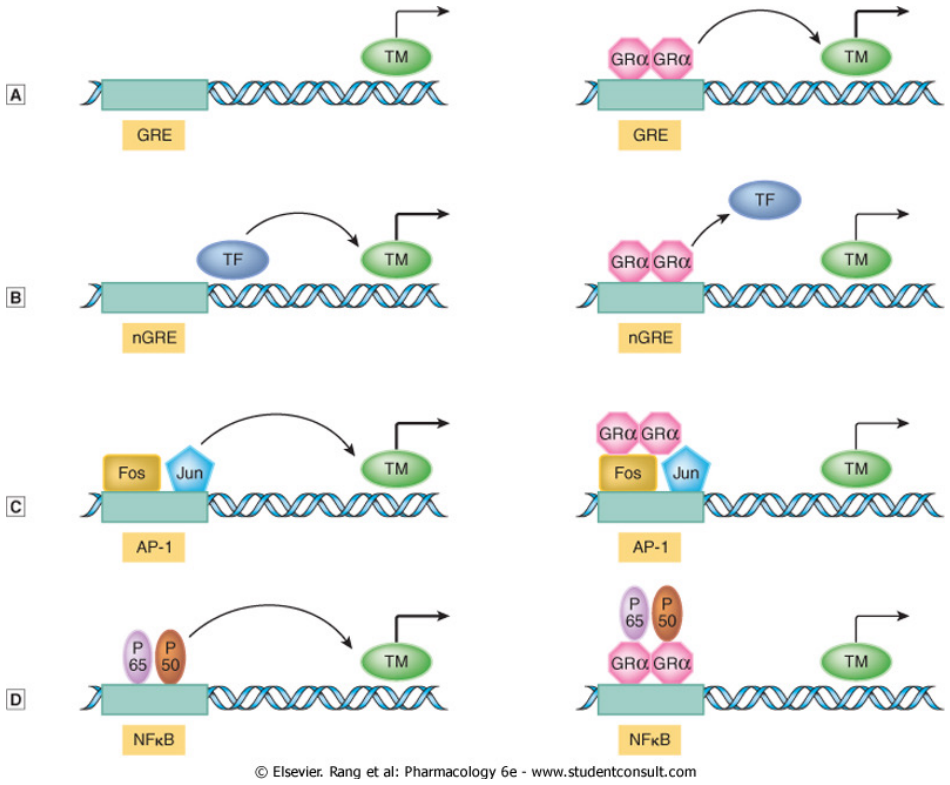
Full length article

Anti-androgen hormonal therapy for cancer and other diseases

Sebastian Student[a,b,*], Tomasz Hejmo[c], Aleksandra Poterała-Hejmo[a,b], Aleksandra Leśniak[d], Rafał Bułdak[c]

GLUCOCORTICOIDS





Activate transcription

Inhibit transcription

Inhibit transcription triggered by Fas/Jun

Inhibit transcription triggered by NF-κB

The use of glucocorticoids in childhood leukemia represented a real therapeutic success story since its introduction 50 years ago.

TARGETING TRANSCRIPTION FACTORS:

Their inhibition (or activation) at the expression level

Their inhibition through physical degradation

Their inhibition (or activation) at the protein/protein interaction level,

Their inhibition (or activation) through the binding of a ligand-based molecule in an activation/inhibition pocket

Their inhibition (or activation) at the protein/DNA binding level

Lambert et al., 2018. Molecules, 23, 1479; doi:10.3390/molecules23061479

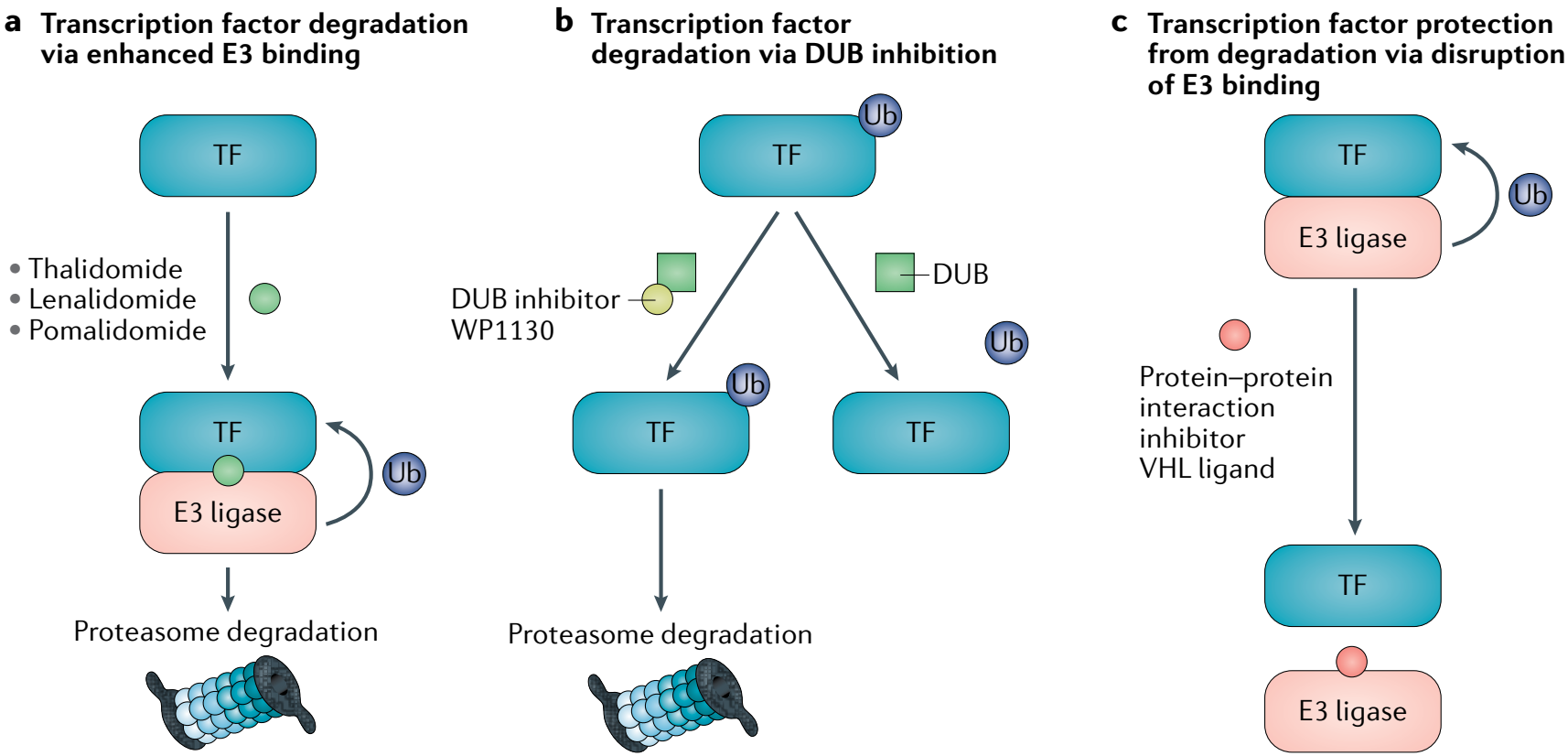


Fig. 4 | **Approaches to modulate transcription factor stability by way of regulating ubiquitylation.**

Bushweller. *Nature Reviews in Cancer* **2019**, *19*; 611.

TARGETING TRANSCRIPTION FACTORS:

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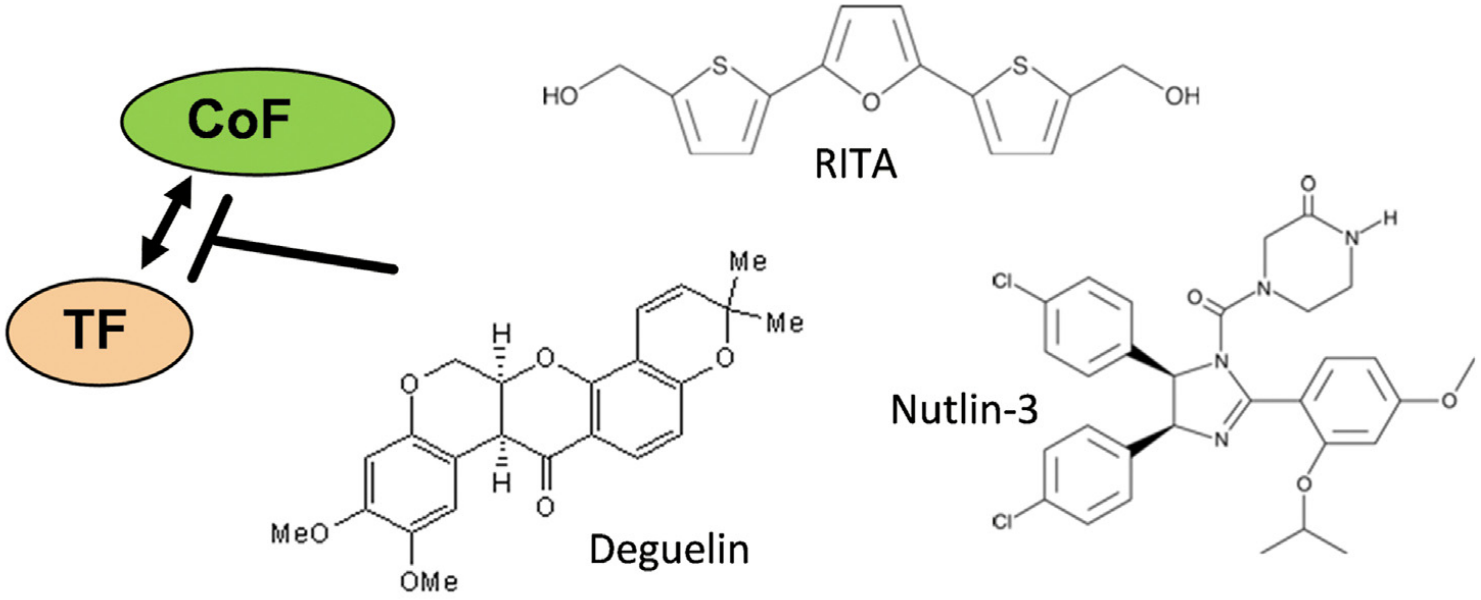
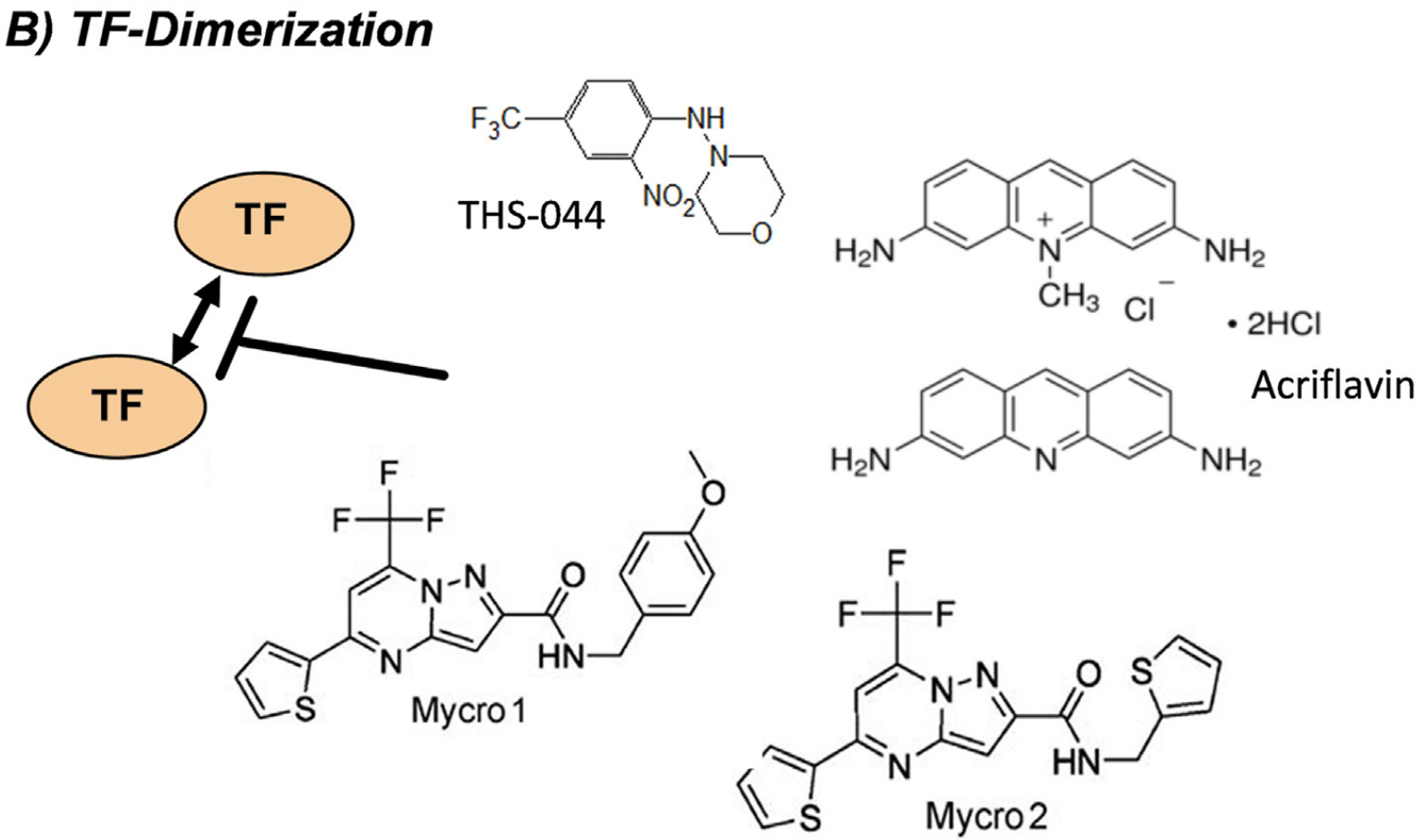
Lambert et al., 2018. Molecules, 23, 1479; doi:10.3390/molecules23061479

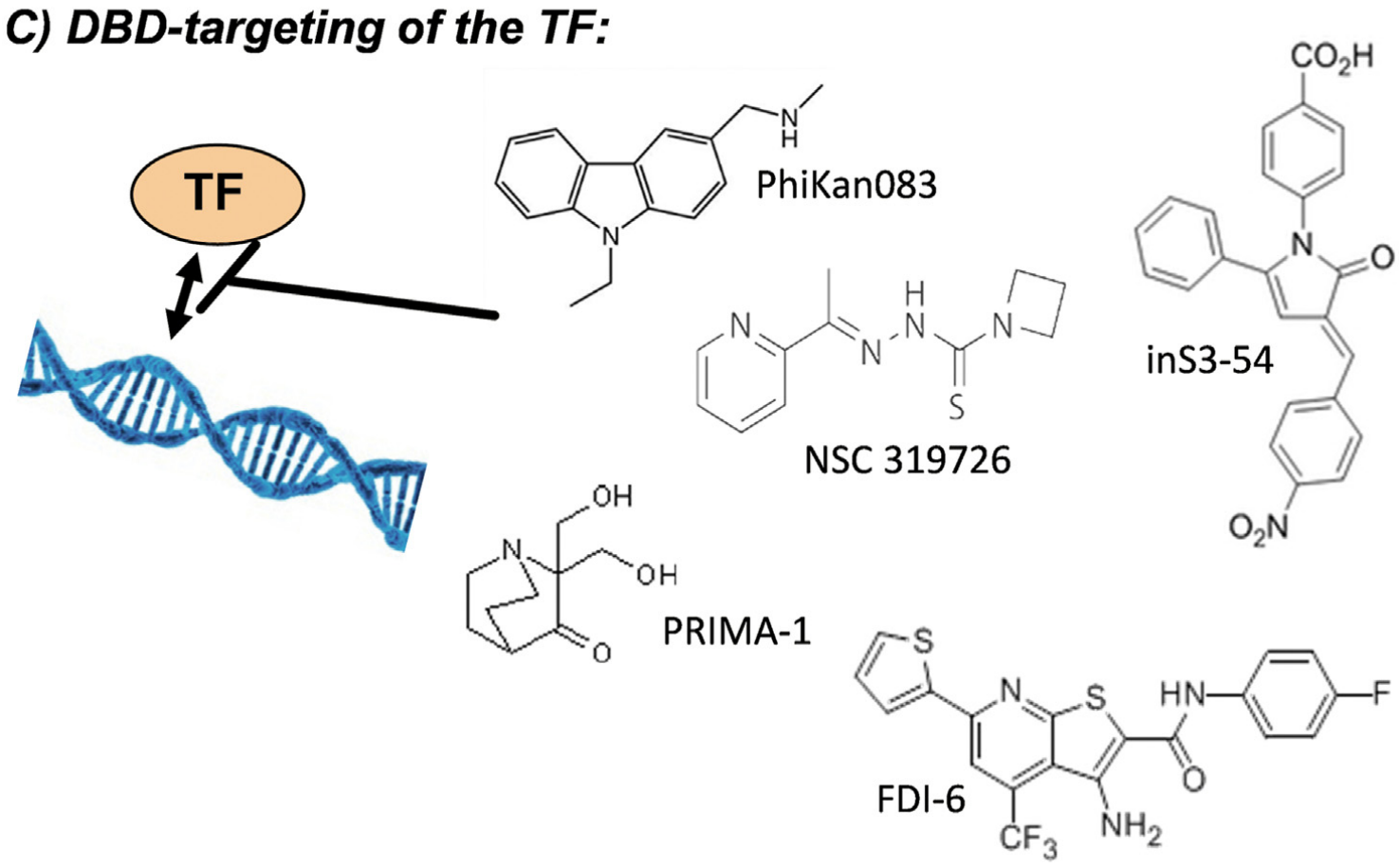
A) Protein-protein interface between TF and Co-factor:



J. Hagenbuchner, M.J. Ausserlechner. Biochemical Pharmacology *107* (2016) 1–713



J. Hagenbuchner, M.J. Ausserlechner. Biochemical Pharmacology *107* (2016) 1–713



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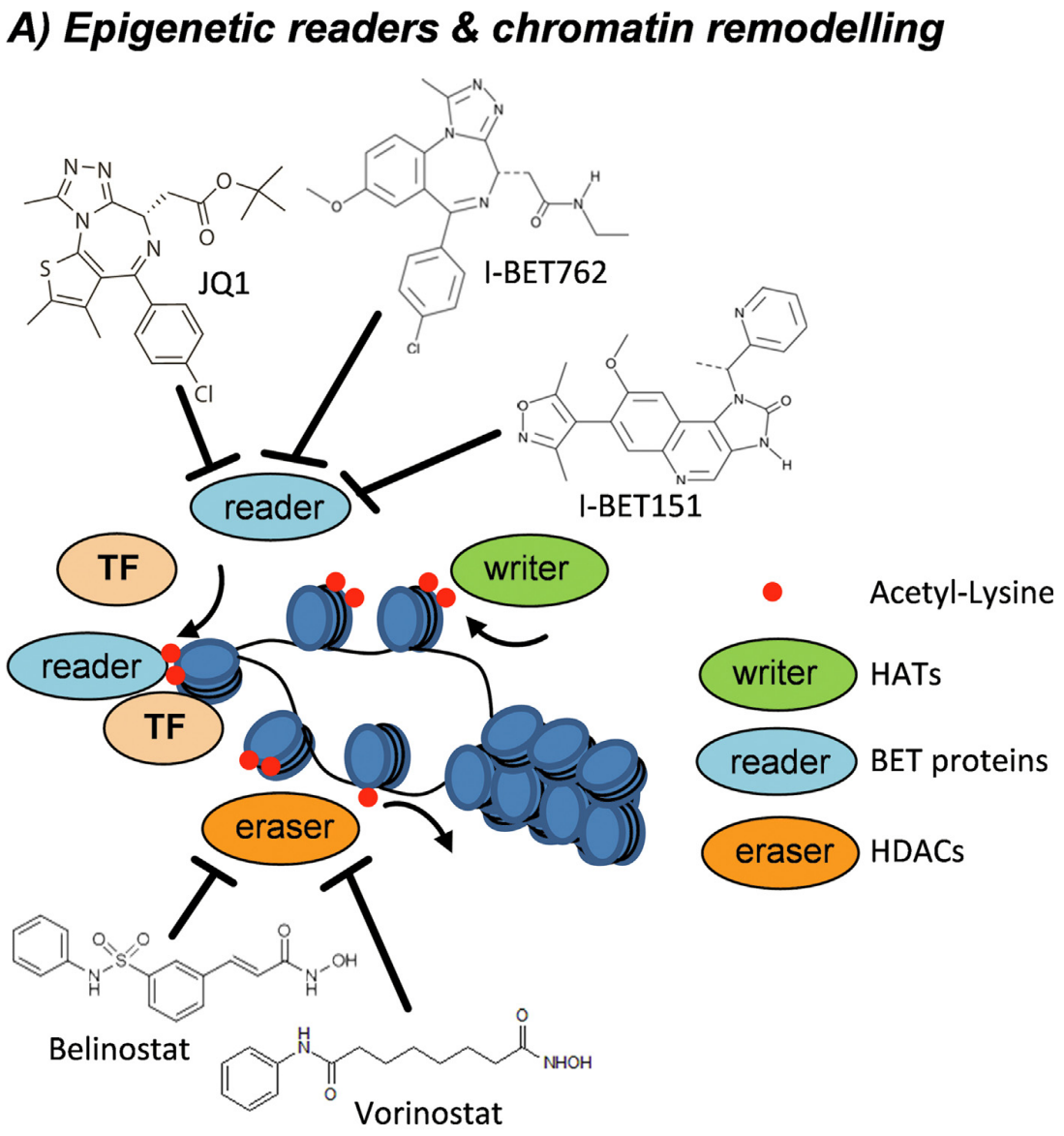
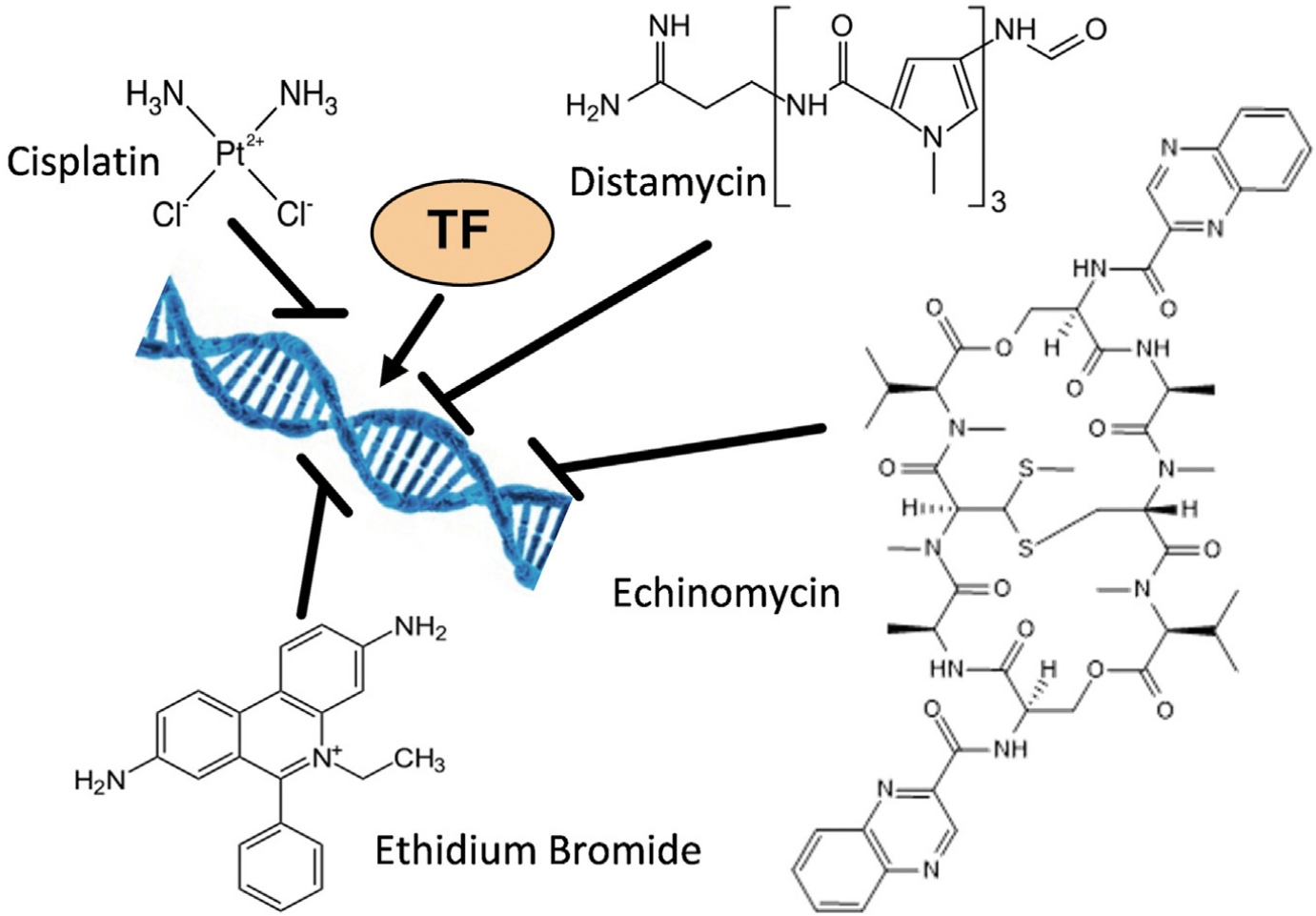


J. Hagenbuchner, M.J. Ausserlechner. Biochemical Pharmacology *107* (2016) 1–713

B) DNA-modifying and intercalating compounds



J. Hagenbuchner, M.J. Ausserlechner. Biochemical Pharmacology *107* (2016) 1–713

TRABECTEDIN AS EXAMPLE

- Trabectedin (Yondelis®, ET-743) : a marine-derived natural product that was initially isolated from the marine ascidian *Ecteinascidia turbinata*
- First evidence since late 1960's
- Structural elucidation in 1990 (Rinehart et al. and Wright et al.)

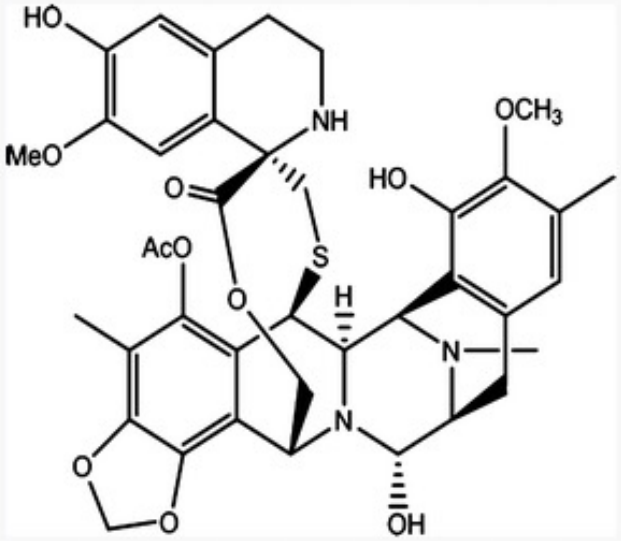


Fig. 1
Chemical structure of trabectedin

Larsen et al., 2015

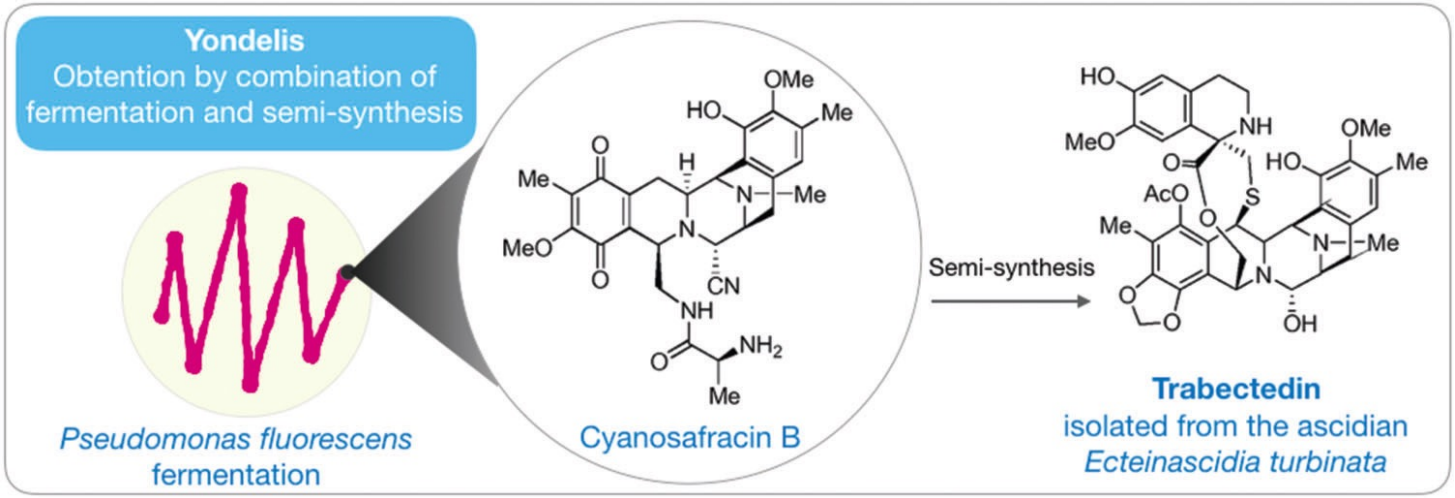
SUPPLY – FIRST BIG PROBLEM!

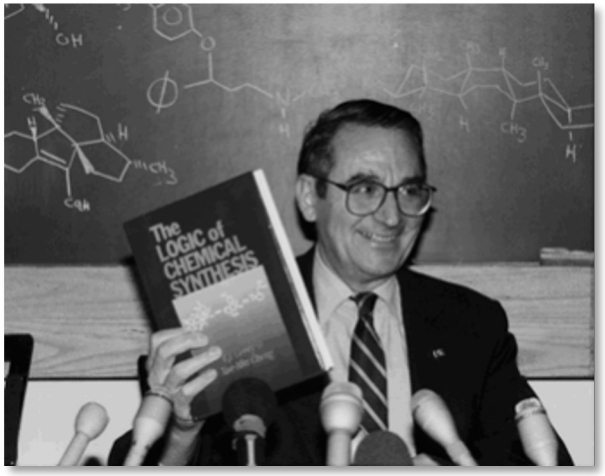
- Yield from native ascidians - 0.0001%
- Aquaculture
- Productivity:
  - 2001 – 80 ton
  - 2004 – 100 ton
  - Max – 250 ton
- Yield:
  - 0.5 and 4.0 µg g^{-1}

Quantity was enough for the clinical trials, BUT not viable for commercial purpose.

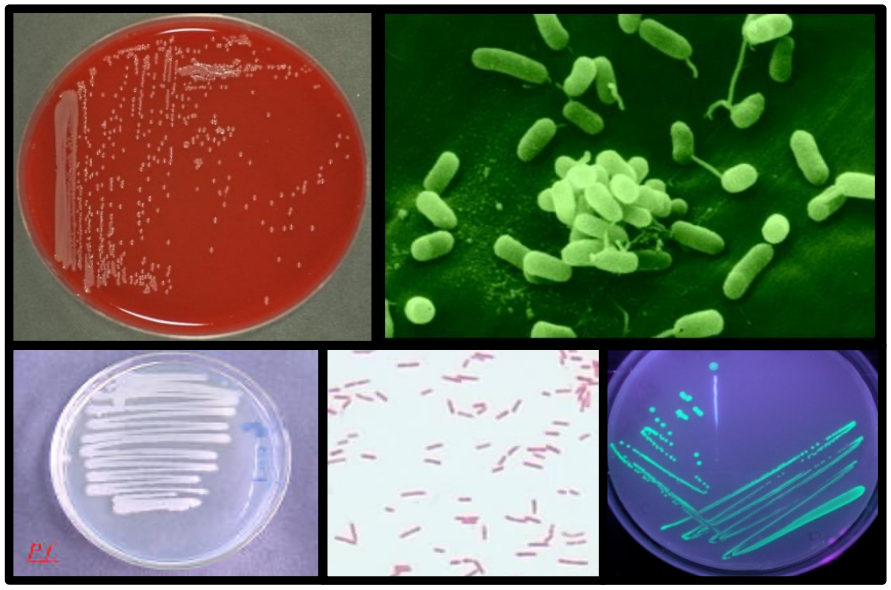
Cuevas & Francesch, 2009. *NPR* 26: 322-337.

SOLUTION





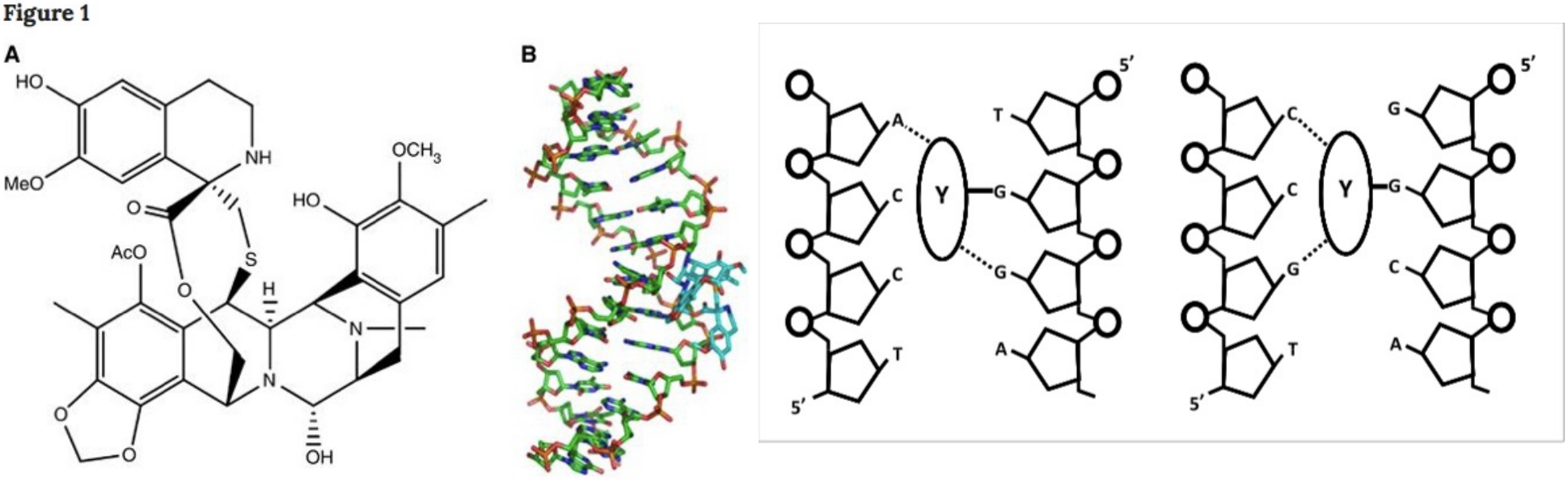
Elias James Corey
Emeritus Professor Harvard U.
Nobel Prize in Chemistry, 1990



Pseudomonas fluorescens

MECHANISM OF ACTION

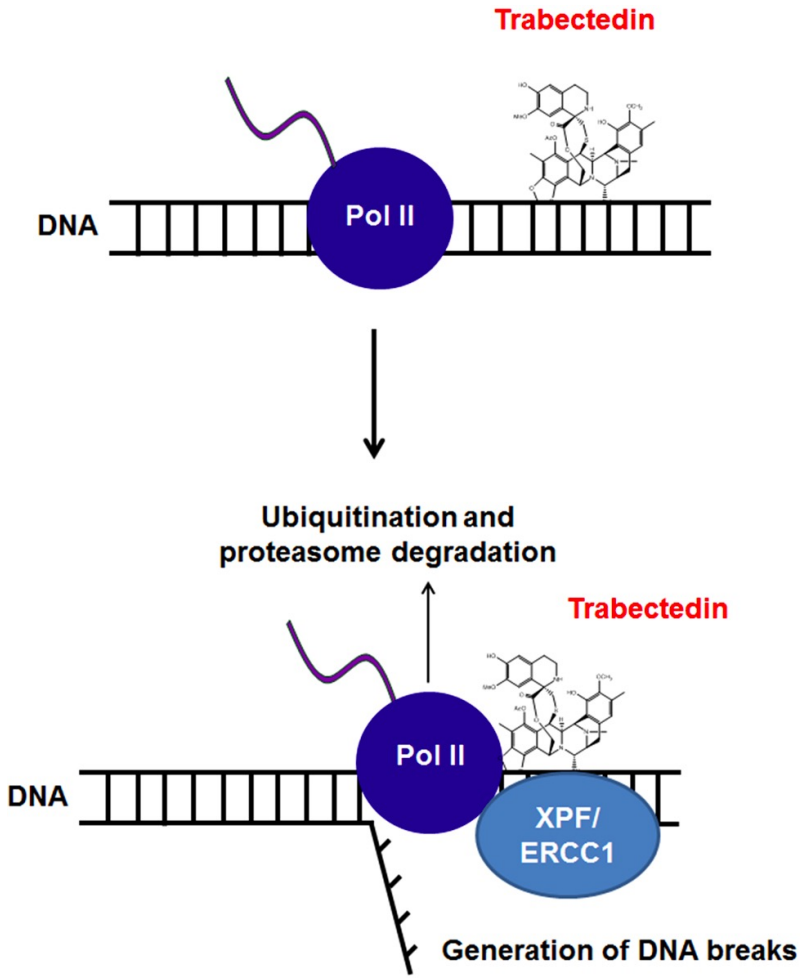
- Bind covalently to the exocyclic amino group of guanines in the minor groove of DNA



D'Inalci et al., 2014, British J Cancer 111: 646

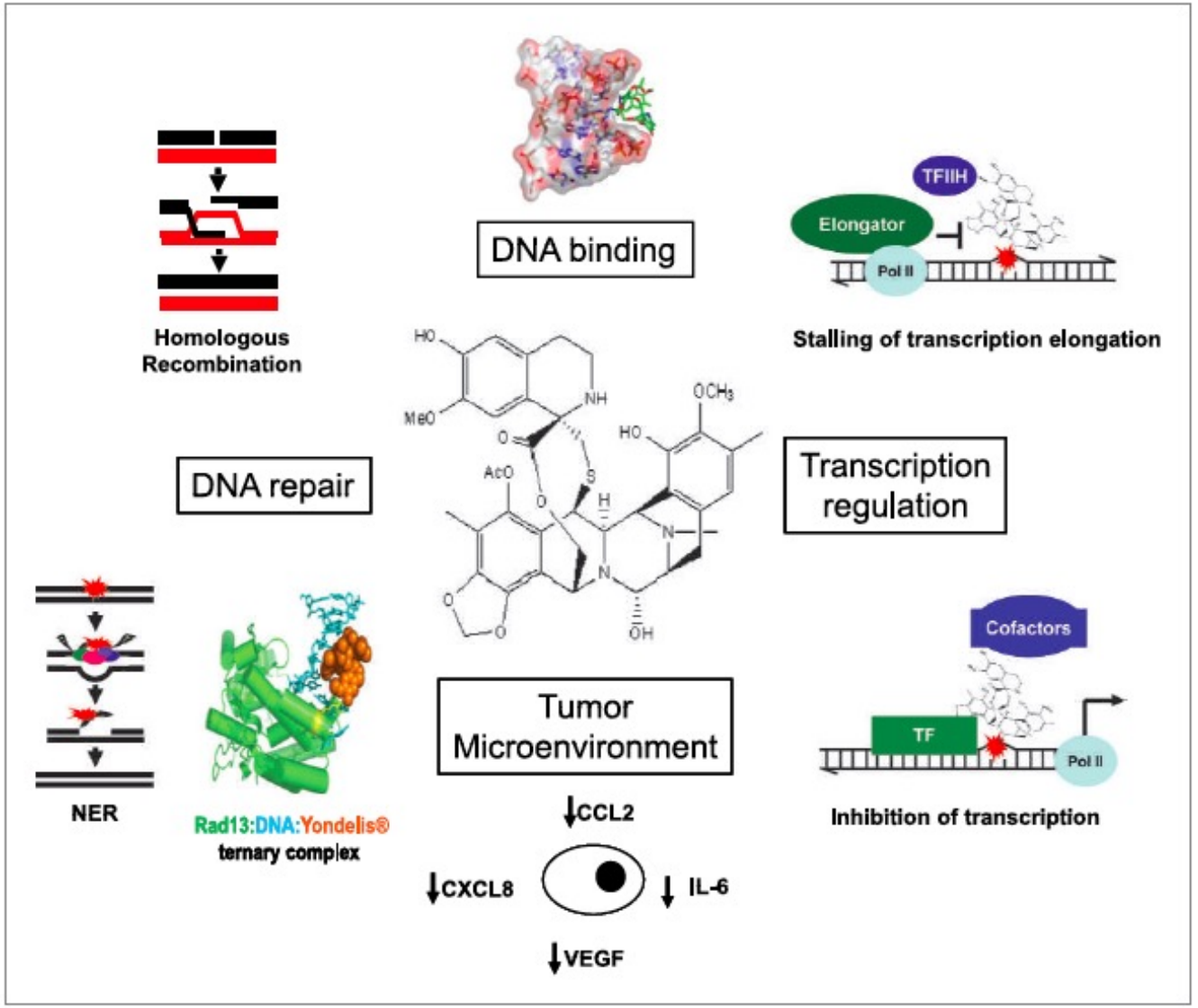
D'Inalci and Galmarini, 2010, Mol Cancer Ther; 9(8); OF1–7.

BLOCKADE AND DEGRADATION OF RNA POLYMERASE II



Larsen et al., Cancer Chemother Pharmacol (2016) 77:663–671

MECHANISM OF ACTION



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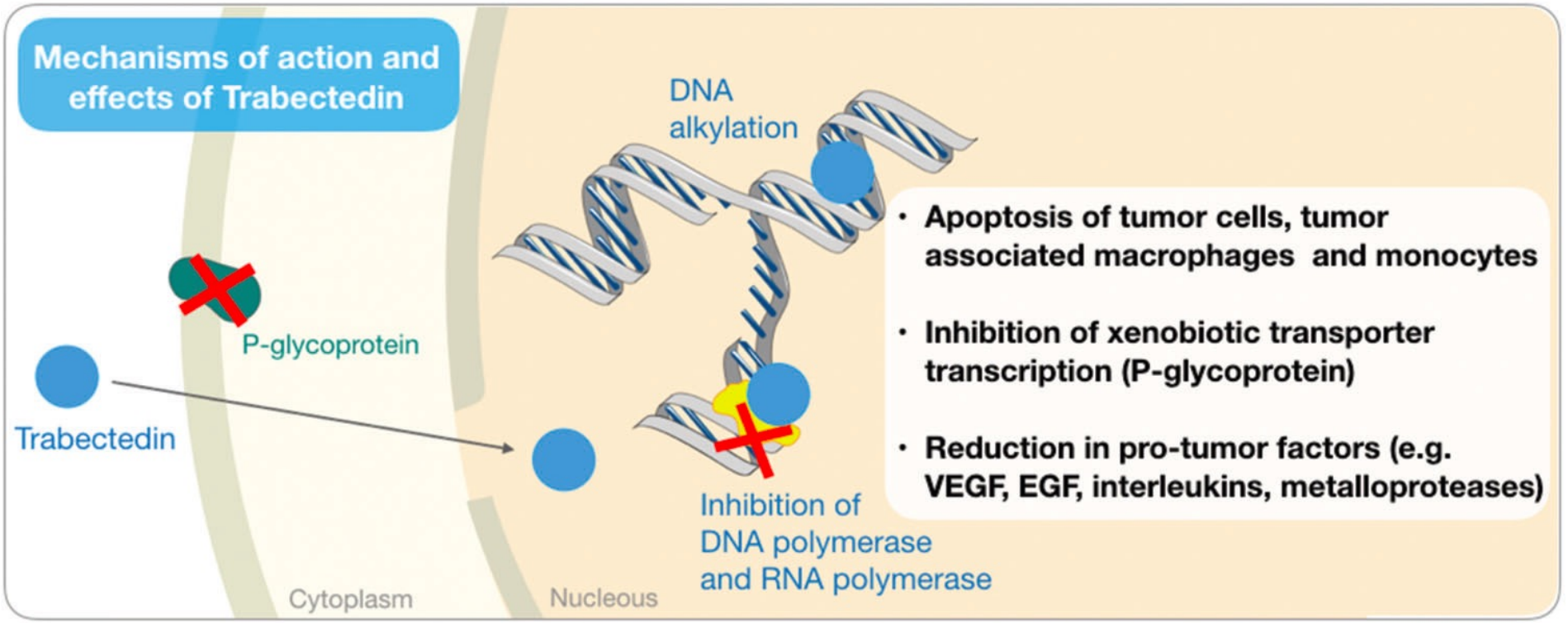


YOUTUBE VIDEO!

Jimenez et al., 2018. Clinics, 73, e482s.

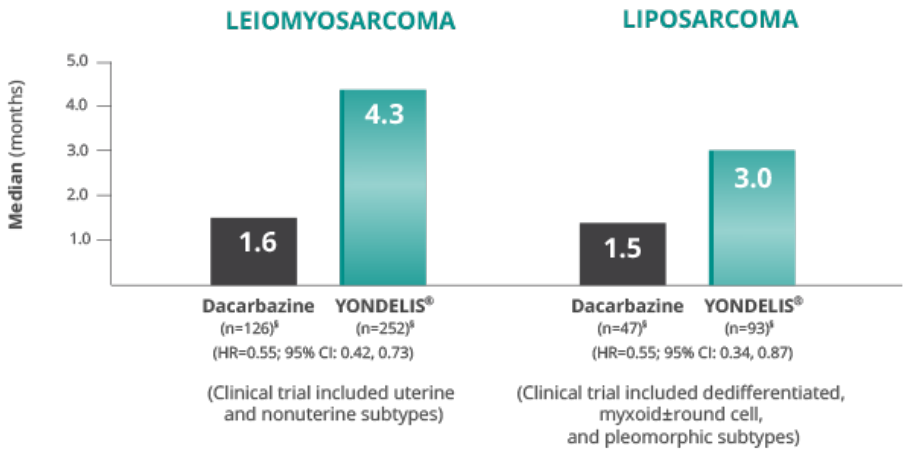
UNIQUE FEATURES:

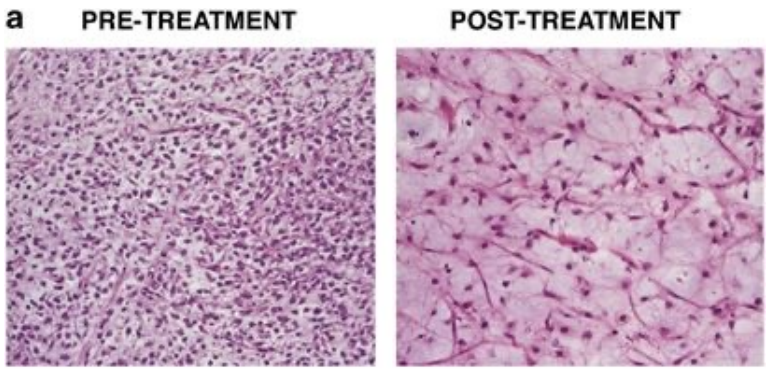
- Trabectedin was the first compound able to displace an oncogenic transcription factor from its target promoters with high specificity.
- Cells deficient in nucleotide excision repair (NER) are generally more sensitive to cisplatin while they are partially resistant to trabectedin
- Cells deficient in homologous recombination (HR) (e.g., with mutations of BRCA1 or BRCA2 genes) are sensitive to trabectedin as well as to platinum compounds

D'Inalci et al., 2014, British J Cancer 111: 646

CLINICAL USES:

- YONDELIS® is the only treatment recently approved specifically for unresectable or metastatic liposarcoma or leiomyosarcoma after an anthracycline-containing regimen
- Presence of the fusion protein FUS-CHOP or EWS-CHOP – acting as abnormal transcrition factors. Trabectedin blocks the trans-activating ability of these chimaeras by displacing the oncogenic fusion protein, inducing rediferenciation.





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