Developmental Therapeutics

New Promising Targets

Cyclin-dependent Kinases (CDKs)

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Conflict of Interest Disclosure

I herewith declare that I have potential conflicts of interest with several pharmaceutical companies, the drugs of which will be mentioned during my presentation, predominantly in form of unrestricted research grants donated to the research institutes directed by me, but also as honoraria for consulting

Background

- The fundamental processes of cell growth and division are governed by the tightly regulated processes that maintain the cell cycle
- The cell cycle progression is regulated and counter-regulated in a highly sophisticated way to guarantee homeostasis despite extra- and intra-cellular disturbances
- The cell cycle progression is positively regulated by a family of protein kinases referred to as cyclin-dependent kinases (CDKs)

Background

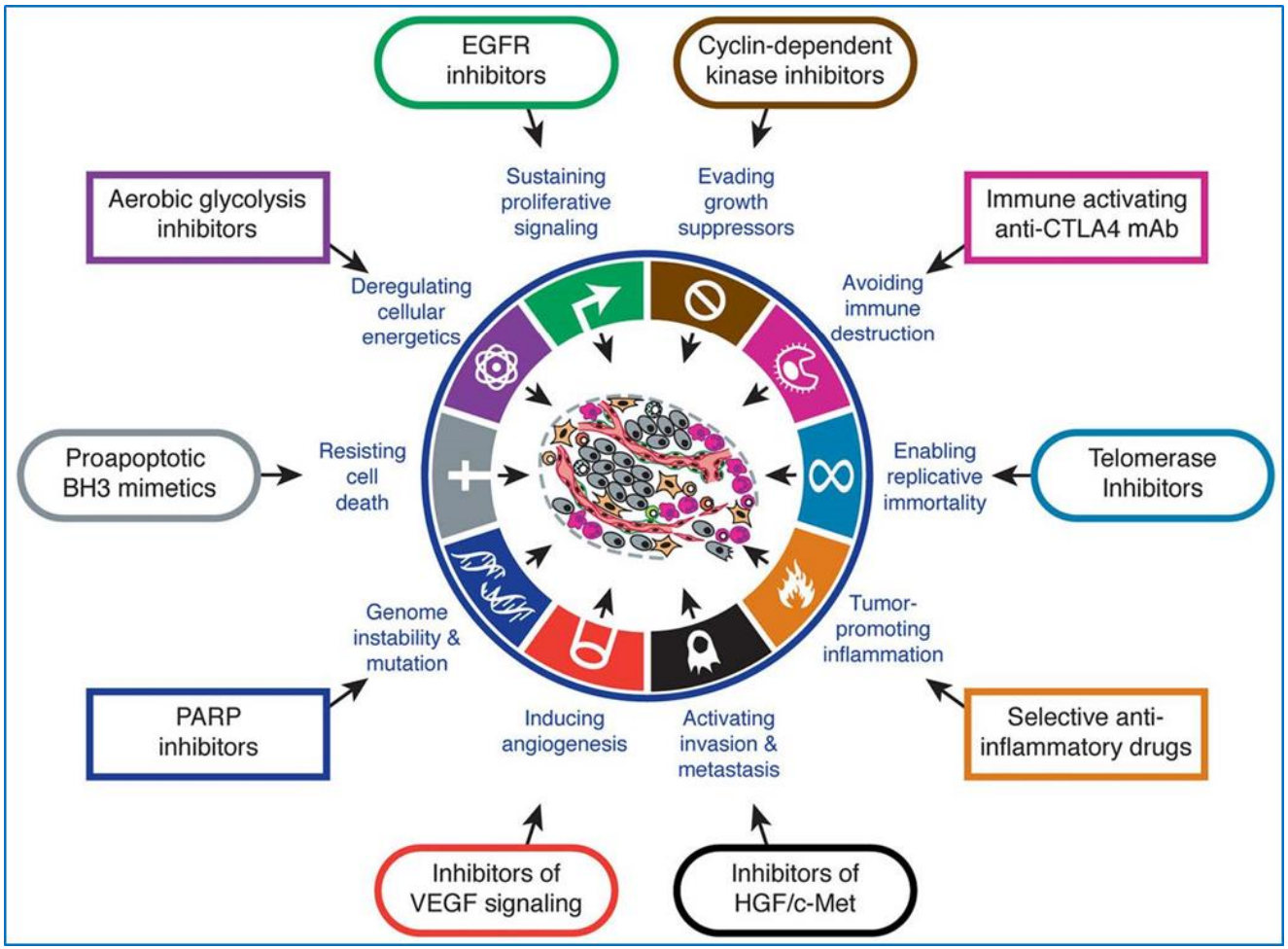
- CDKs are a class of serine/threonine kinases
- They consist of a catalytic subunit (CDK1-9) which transfers phosphate to appropriate substrates and form a complex with a regulatory subunit, the cyclin (A-T), to get enzymatically active
- The cyclins are expressed in response to growth-factor signaling, whereas the CDKs are constitutively expressed
- CDKs are subject to direct regulation by small polypeptide inhibitory proteins (CKIs). There exist two distinct families of natural CKIs, the INK family ($p16^{Ink4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$, $p19^{Ink4d}$) and the Cip/Kip family (p21, p27, p57)

Targeting the Cell Cycle

- Tubulin or microtubules
- Mitotic kinases
  - Kinesins
  - Polo-like kinases
  - Aurora kinases
- Cyclin-dependent kinases (CDKs)
  - Chemical inhibition of CDK catalytic activity
  - Inhibition of interaction between cyclins and CDKs
  - Decreasing cyclin expression
  - Promotion of degradation of cyclins by phosphorylation
  - Restauration of endogenous CDK inhibitor function
- Checkpoint kinases

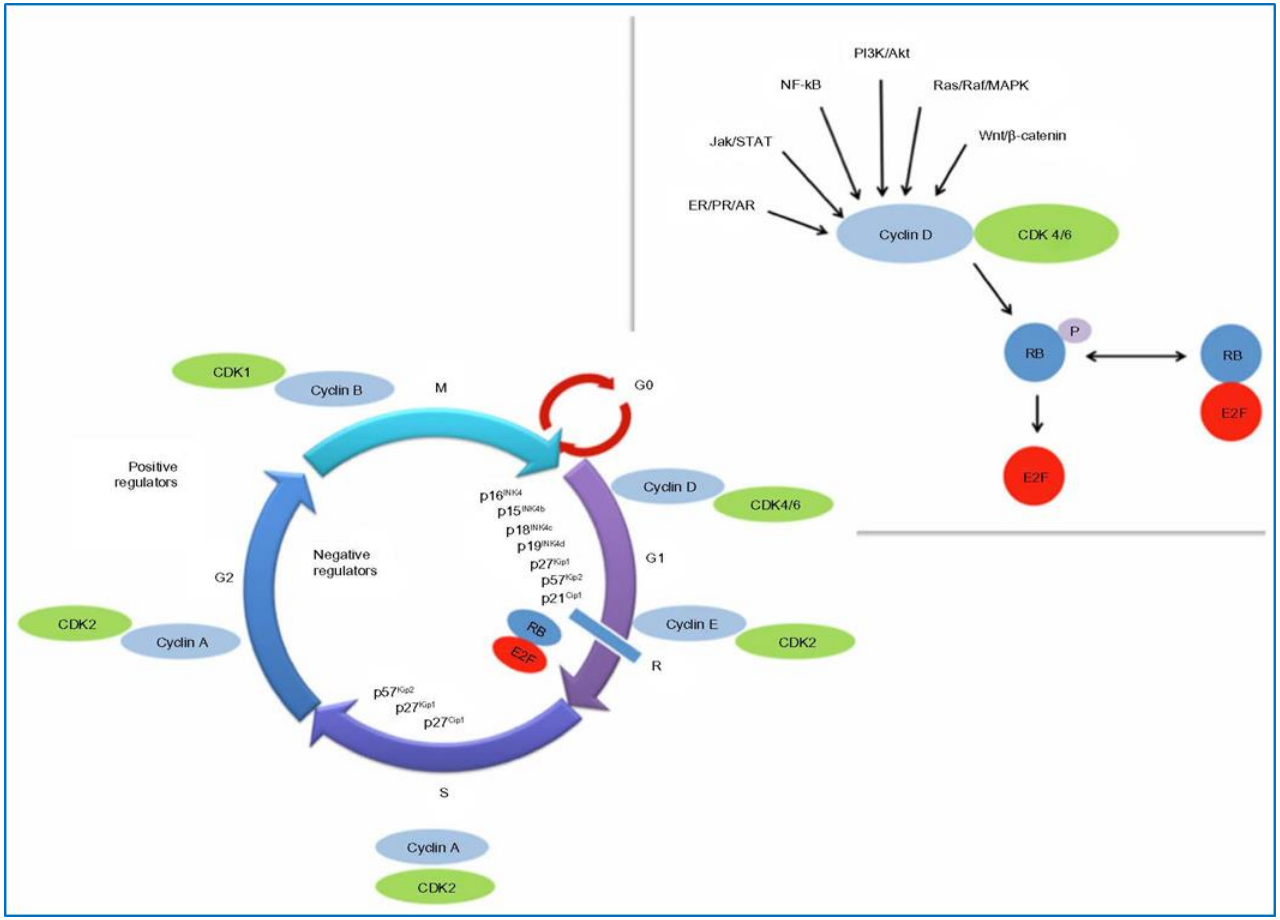
Adapted from
Benson et al; Br J Cancer 92:7-12,2005

Therapeutic Targeting of the Hallmarks of Cancer



Hanahan & Weinberg; Cell 144:646-674,2011

The Cell Cycle and Regulatory Process



Cadoo et al;
Breast Cancer: Targets and Therapy 6:123-133,2014

Dysregulation of the Cyclin D - CDK4/6 - INK4 - Rb Pathway in Cancer

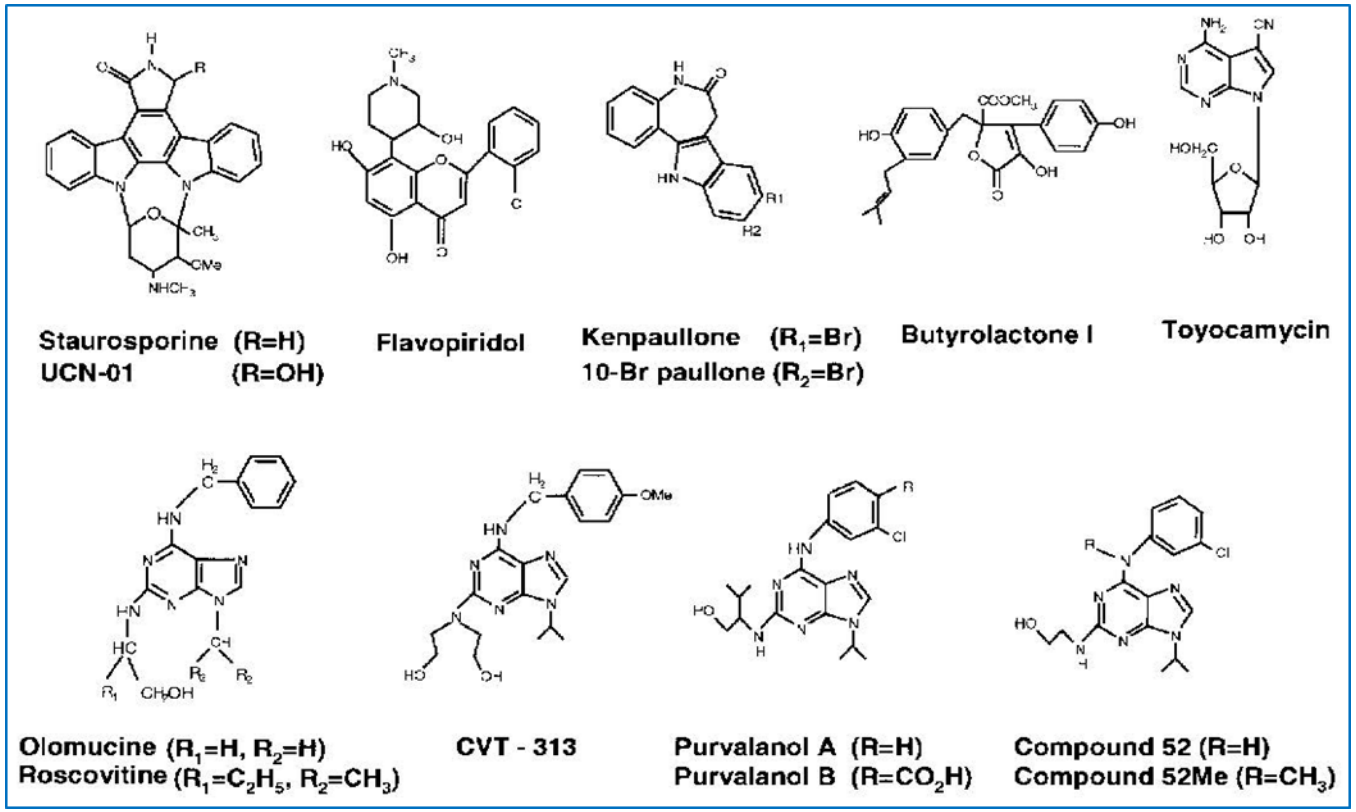
- Cyclin D - CDK4/6 - INK4 - Rb pathway
  Disturbence / hyperactivity in cancer → cell cycle progression
  - Overexpression / amplification / translocation of cyclin D genes
  - Mutations and amplification of CDK4/6
  - Loss of CDK4/6 inhibition, such as INK4 protein
- Rb loss →
  - Disruption of cell cycle
  - CDK4/6 independence
  - Resistance to CDK4/6 inhibition
- Rb+ cancers →
  - CDK4/6 dependent

Cyclin D1 Deregulation in Cancer

Mechanism of deregulation	Tumour type	Frequency
Amplification and overexpression		
CCND1 amplification	Head and neck squamous cell carcinoma	26–39%
Cyclin D1 overexpression	Head and neck squamous cell carcinoma	20–68%
CCND1 amplification	Non-small-cell lung cancer	5–30%
Cyclin D1 overexpression	Non-small-cell lung cancer	18–76%
CCND1 amplification	Endometrial cancer	26%
Cyclin D1 overexpression	Endometrial cancer	40–56%
CCND1 amplification	Melanoma	0–25%
Cyclin D1 overexpression	Melanoma	30–65%
CCND1 amplification	Pancreatic cancer	25%
Cyclin D1 overexpression	Pancreatic cancer	42–82%
CCND1 amplification	Breast cancer	15–20%
Cyclin D1 overexpression	Breast cancer	50–70%
CCND1 amplification	Colorectal cancer	2.5%
Cyclin D1 overexpression	Colorectal cancer	55%
Chromosomal rearrangement and overexpression		
CCND1: *IGH* translocation t(11;14)(q13;q32)	Mantle cell lymphoma	>90%
Cyclin D1 overexpression	Mantle cell lymphoma	>90%
CCND1: *IGH* translocation t(11;14)(q13;q32)	Multiple myeloma	16%
Cyclin D1 overexpression	Multiple myeloma	30–50%
Splice variants and transcript aberrations		
3′ UTR rearrangements, microdeletions or point mutations	Mantle cell lymphoma	4–10%
Cyclin D1b overexpression	Breast cancer	22%*
Cyclin D1b overexpression	Prostate cancer	27%*
Mutations affecting nuclear export and proteolysis		
Cyclin D1 T286R; Δ266–295	Oesophageal cancer	4%
Cyclin D1 P287S; P287T; Δ289–292	Endometrial cancer	4%
FBXO4 S8R, S12L, P13S, L23Q, G30N and P76T	Oesophageal cancer	14%

Musgrove et al; Nature Reviews Cancer 11:558-572,2011

Chemical Structure of Small Molecular CDK Inhibitors

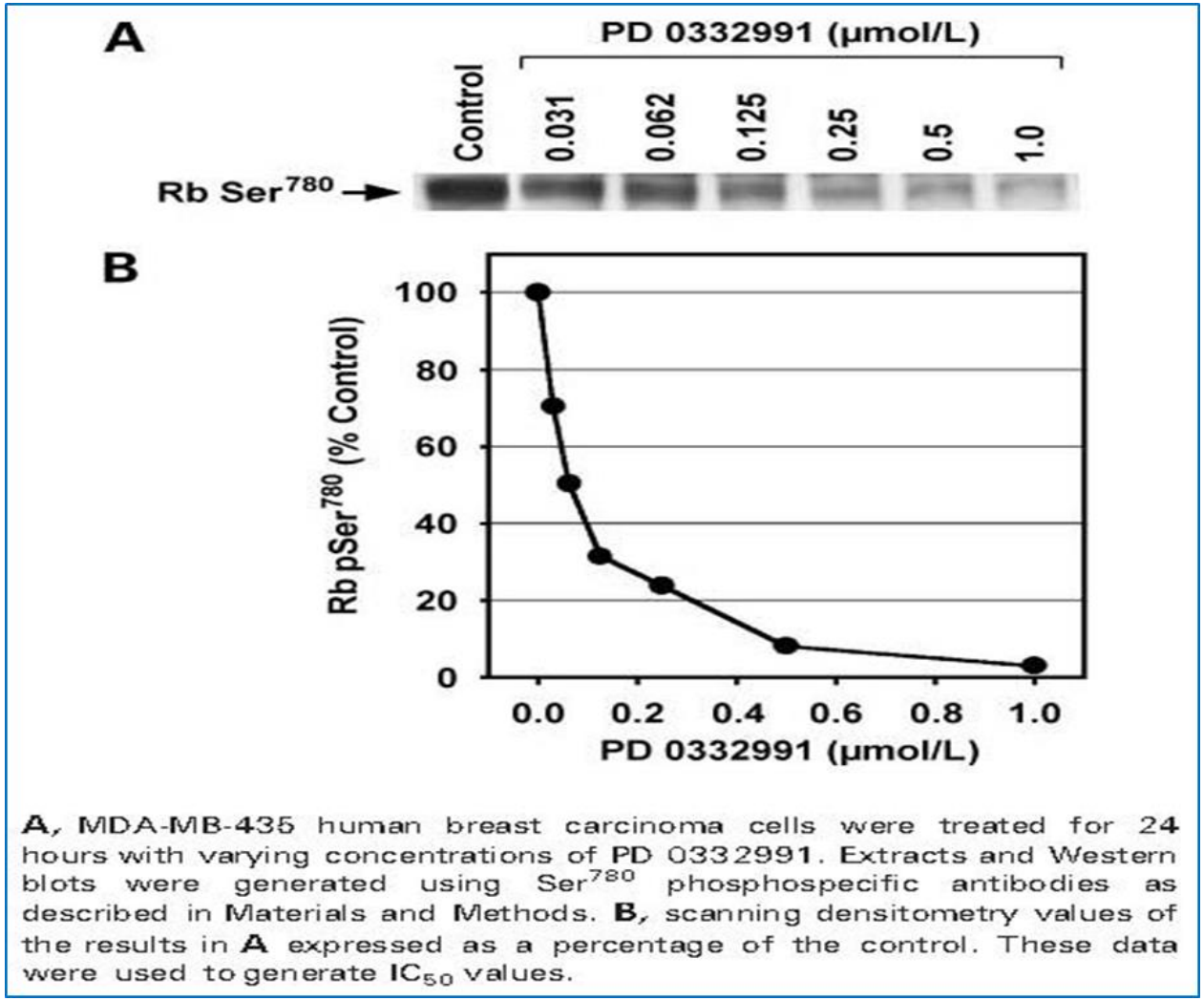


Summary. With knowledge of the role of CDKs in cell cycle regulation and the discovery that approximately 90% of all neoplasias are associated with “CDK hyperactivation” leading to the inactivation of the Rb pathway, novel CDK inhibitors are being developed.

Future clinical trials should determine what is the best schedule for administering chemical CDK inhibitors, should determine what is the best combination of chemical CDK inhibitors and standard chemotherapeutic agents, and should demonstrate CDK modulation in tumor samples from patients treated with CDK inhibitors.

Senderowicz & Sausville;
J Natl Cancer Inst 92:376-387,2000

Inhibition of Rb Phosphorylation at Ser^{780} by PD 0332991



A, MDA-MB-435 human breast carcinoma cells were treated for 24 hours with varying concentrations of PD 0332991. Extracts and Western blots were generated using Ser^{780} phosphospecific antibodies as described in Materials and Methods. **B,** scanning densitometry values of the results in **A** expressed as a percentage of the control. These data were used to generate IC_{50} values.

Fry et al; Mol Cancer Ther 3:1427-1437,2004

Molecular Alterations Involving the Rb Pathway in Breast Cancer Subtypes

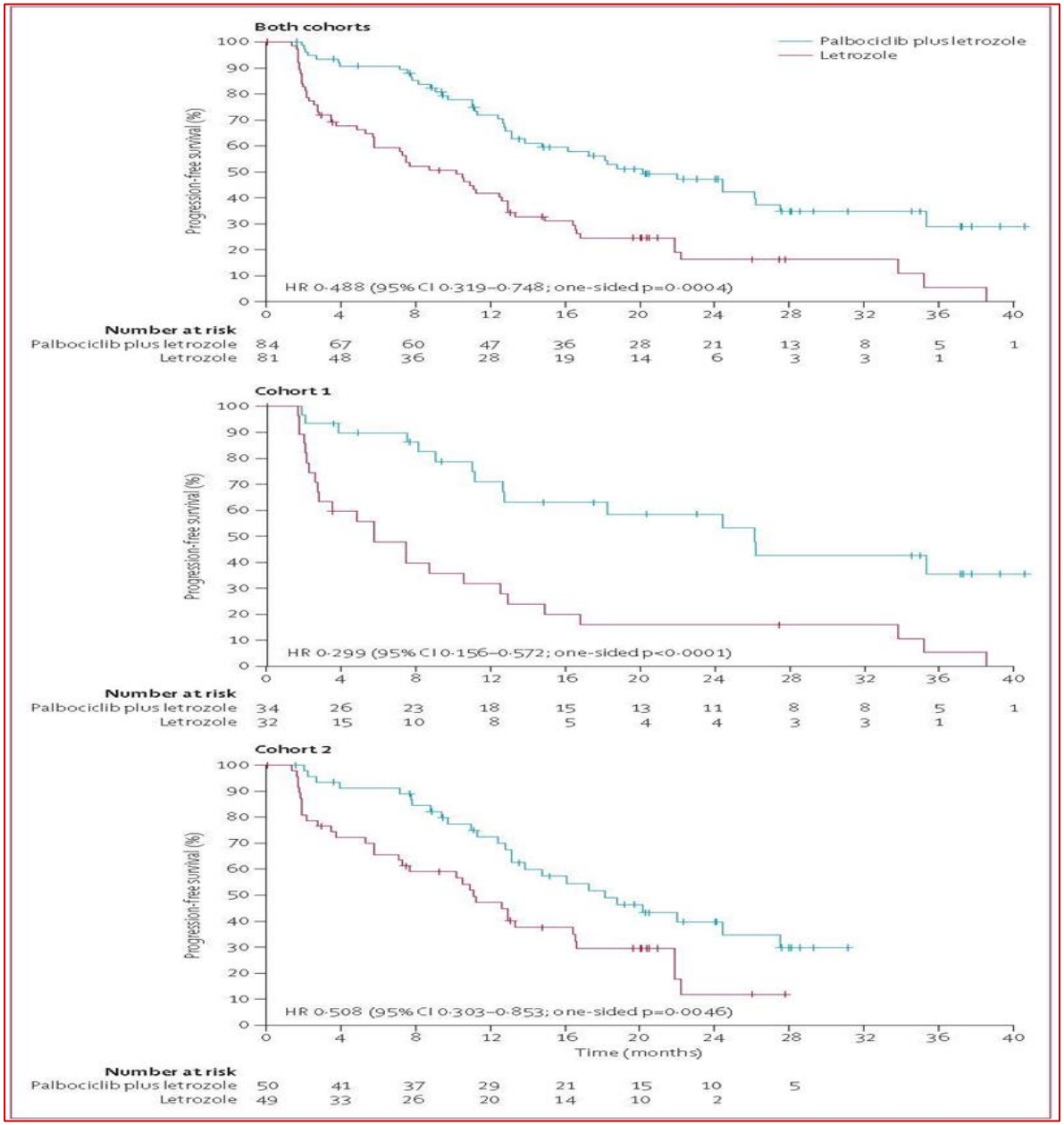
	Luminal A	Luminal B	HER2-enriched	Basal-like
Cyclin D1 amplification	29%	58%	38%	9%
CDK4 gain	14%	25%	24%	
RB1 mutation/loss				20%
Other	Low expression of p18/high expression of RB1	High FOXM1		High expression of p16/low expression of RB1

Migliaccio et al; Curr Opin Oncol 26:568-575,2014

Randomized Phase II-Study of Palbociclib in Combination with Letrozole vs Letrozole Alone for First-Line Treatment of ER+/HER2- Advanced Breast Cancer (PALOMA-1/TRIO-18)

(N=81) Letrozole 2.5mg continuously	Randomization Cohort 1: ER+/HER2- Cohort 2: +CCND1 amplified and/or loss of p16	(N=84) Letrozole + palbociclib 125mg QD: 3 wks on–1 wk off
	Progression-free Survival	
10.2 months	HR 0.488 (95%CI; 0.319 to 0.748; p=0.0004)	20.2 months
5.7 months	Cohort 1: N=66; HR 0.299 (95%CI; 0.156 to 0.572; p=0.0001)	26.1 months
11.1 months	Cohort 2: N=99; HR 0.508 (95%CI; 0.303 to 0.853; p=0.0046)	18.1 months
	Overall Survival	
33.3 months	HR 0.813 (p=0.42)	37.5 months
1% patients	G3/4 Neutropenia	54% patients
0% patients	Leucopenia	19% patients
2% patients	Discontinuation due to AE	13% patients

Finn et al; Lancet Oncol 16:25-35,2015



PALOMA-1/TRIO-18 Progression-free Survival

Finn et al; Lancet Oncol 16:25-35,2015

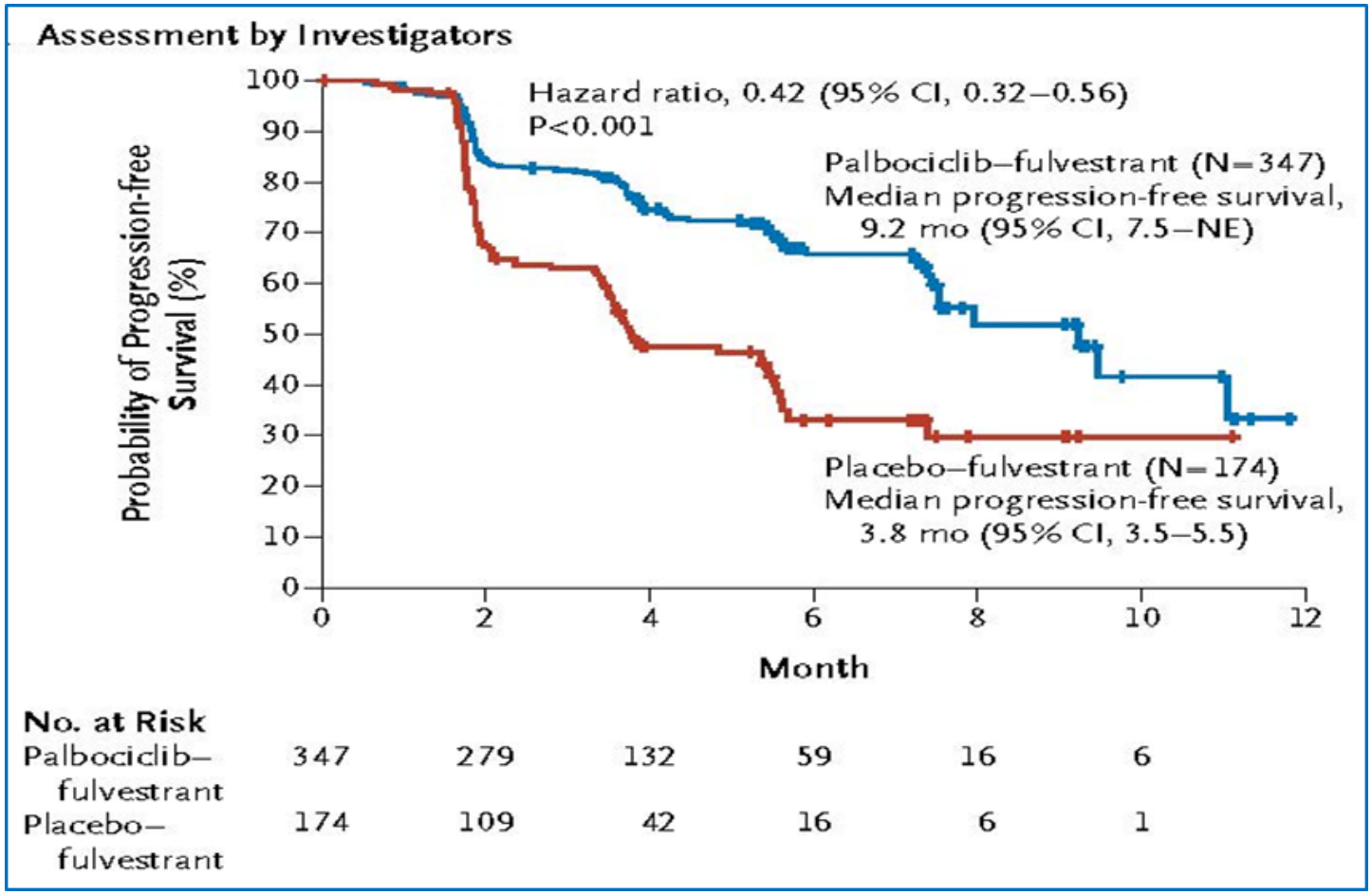
PALOMA-3
Palbociclib plus Fulvestrant vs Placebo plus Fulvestrant

- Pre-/peri-/post-menopausal breast cancer patients
- Endocrine resistant patients

9.2 months	Progression-free Survival HR 0.42 (95%CI; 0.32 to 0.56; p<0.001)	3.8 months
	G3/4 Adverse Events	
62.0%	Neuropenia	0.6%
25.2%	Leucopenia	0.6%
2.6%	Anemia	1.7%
2.3%	Thrombocytopenia	0.0%
2.0%	Fatigue	1.2%
2.6%	Discontinuation due to AE	1.7%

Turner et al; New Engl J Medicine 373:209-219,2015

PALOMA-3
Progression-free Survival



Turner et al; New Engl J Medicine 373:209-219,2015

Ribociclib / Abemaciclib in Breast Cancer

MONALEESA-2		
- Postmenopausal - HR positive - HER2 negative - Locally advanced, inoperable or metastatic - First-line treatment	Ribociclib + letrozole vs Placebo + letrozole	Primary endpoint - PFS Secondary endpoints - OS - ORR, ECOG PS, QoL - Safety, PK
MONARCH-3		
- Postmenopausal - HR positive - HER2 negative - Locally advanced, inoperable or metastatic - First-line treatment	Abemaciclib + Anastrazole or letrozole vs Placebo + Anastrazole or letrozole	Primary endpoint - PFS (local) Secondary endpoints - OS - ORR, DOR, DCR - CBR, PK - Safety
MONALEESA-3		
- Postmenopausal - HR positive - HER2 negative - No or only one prior endocrine treatment	Ribociclib + fulvestrant vs Placebo + fulvestrant	Primary endpoint - PFS (local) Secondary endpoints - OS, PFS (central), ORR - ECOG PS, PRO/QoL - Safety/tolerability
MONARCH-2		
- Postmenopausal - HR positive - HER2 negative - Locally advanced, inoperable or metastatic - No endocrine pretreatment or endocrine primarily or secondarily resistant	Abemaciclib + fulvestrant vs Placebo + fulvestrant	Primary endpoint - PFS Secondary endpoints - OS - ORR, CBR, QoL - Safety, PK

Adjuvant Therapy with Palbociclib in Breast Cancer

PENELOPE		
- High risk: CPS-EG ≥ 3 - ER positive - Pre-/postmenopausal - Completed taxane-based neo-adjuvant therapy, surgery, radiotherapy	Palbociclib + SOC endocrine treatment vs Placebo + SOC endocrine treatment	Primary endpoint - iDFS Secondary endpoints - OS, DDFS, LRFS Strata: - LNN status - Age - Biomarkers (Ki67, pRb, Cyclin D) - Region
PALLAS		
- HR positive - HER2 negative - Stage II or III - Pre-/postmenopausal - Completed neo-adjuvant therapy	Palbociclib (2 years) + SOC endocrine treatment (5+ years) vs Placebo + SOC endocrine treatment (5+ years)	Primary endpoint - iDFS Secondary endpoints - OS, DDFS, LRFS Strata: - Stage - Neo-/adjuvant therapy - Age - Region

Clinical Trials Investigating Selective CDK4/6 Inhibitors in Breast Cancer

Inhibitor	Identifier	Status	Phase	Arms	Tumor type	Biomarkers	Comments
Palbociclib	NCT01684215	Active, not recruiting	1/2	Phase 1: palbociclib or palbociclib and Let Phase 2: palbociclib and Let	AST (phase 1) ER+ HER2− ABC (phase 2)	No	Japanese population
	NCT00141297	Active, not recruiting	1	Palbociclib	AST, including breast	Rb (IHC)	
	NCT01976169	Active, recruiting	1b	Palbociclib and TDM-1	HER2+ ABC	Rb, p16 (IHC)	Pretreated with trastuzumab or other anti-HER2
	NCT01723774	Recruiting	2	Palbociclib and Ana (± Gos if premenopausal)	ER+ HER2− EBC or LABC	No	Neoadjuvant
	NCT01864746 (PENELOPE B)	Recruiting	3	Palbociclib and hormonal therapy (Tam/Gos/aromatase inhibitors) vs. placebo and hormonal therapy	ER+ HER2− with residual after neoadjuvant CHT	No	Adjuvant pRb and cyclin D1 stratification factors
	NCT01740427 (PALOMA2)	Recruiting	3	Palbociclib and Let vs. placebo and Let	ER+ HER2− ABC	No	First-line; aromatase-inhibitor-resistant patients excluded
	NCT02028507 (PEARL)	Recruiting	3	Palbociclib and Exe vs. capecitabine	ER+ HER2− ABC	No	First and second line; NS-aromatase-inhibitor-resistant patients only
	NCT00721409 (PALOMA1)	Active, not recruiting	1/2	Phase 1: palbociclib and Let Phase 2: palbociclib and Let vs. placebo and Let	ER+ HER2− ABC	CCND1 (FISH), p16 (IHC)	
	NCT02040857	Recruiting	2	Palbociclib and hormonal therapy (Tam/Let/Ana/Exe)	ER+ HER2− stage II/III (no T2N0)	No	
	NCT01942135 (PALOMA3)	Recruiting	3	Palbociclib and Ful vs. placebo and Ful	ER+ HER2− ABC	No	First or second line Endocrine pretreated only
	NCT01037790	Recruiting	2	Palbociclib	AST, including breast	Rb (IHC)	
	NCT01320592	Recruiting	1	Palbociclib and paclitaxel	ABC	No	
	NCT01709370	Recruiting	2	Palbociclib and Let	ER+ HER2− >2 cm	No	Neoadjuvant
	NCT02008734	Active, not recruiting	2	Palbociclib vs. no treatment	ER+ HER2− ECB (≥1.5 cm, G3 or Ki67 ≥20%)	No	Presurgery, treatment duration 14–21 days
LEE011	NCT02088684	Active, not yet recruiting	1b/2	Phase 1b: LEE011 and Ful or LEE011 and BKM120 and Ful or LEE011 and BYL719 and Ful Phase 2: LEE011 and Ful vs. LEE011 and BKM120 and Ful vs. LEE011 and BYL719 and Ful	ER+ HER2− ABC	No	Any line of prior hormonal therapy Phase 1: up to 2 line of CHT allowed Phase 2: only 1 line of CHT allowed

Migliaccio et al; Curr Opin Oncol 26:568-575,2014

Clinical Trials Investigating Selective CDK4/6 Inhibitors in Breast Cancer

Inhibitor	Identifier	Status	Phase	Arms	Tumor type	Biomarkers	Comments
	NCT01872260	Recruiting	1b/2	Phase 1b: LEE011 and Let or BYL719 and Let or LEE011 and BYL719 and Let Phase 2: LEE011 and Let vs. BYL719 and Let vs. LEE011 and BYL719 and Let	ER$^+$ HER2$^-$ ABC	No	Phase 1: any line of prior hormonal therapy, only 1 line of CHT allowed Phase 2: first line
	NCT01237236	Active, not recruiting	1	LEE011	AST, including breast	No	
	NCT01857193	Recruiting	1b/2	Phase 1b: LEE011 and everolimus and Exe or LEE011 and Exe Phase 2: LEE011 and everolimus and Exe or LEE011 and Exe vs. everolimus and Exe	ER$^+$ HER2$^-$ ABC	No	Patients recurring while on, or within 12 months of end of adjuvant Let or Ana or progressing on or within 1 month from the end of Let or Ana for ABC
	NCT01958021 (MONALEESA2)	Recruiting	3	LEE011 and Let vs. placebo and Let	ER$^+$ HER2$^-$ ABC	No	First line
	NCT01919229 (MONALEESA1)	Recruiting	2	LEE011 (400 mg) and Let vs. LEE011 (600 mg) and Let vs. Let	ER$^+$ HER2$^-$ EBC ($\geq$1 cm, G2 or G3)	No	Presurgery Treatment duration 14 days
	NCT01898845	Recruiting	1	LEE011	AST, including breast	No	Asian patients
	NCT02154776	Active, not yet recruiting	1	LEE011 and BKM120 and Let	ER$^+$ HER2$^-$ ABC	No	
Abemaciclib	NCT02014129	Recruiting	1	Abemaciclib	AST, including breast	No	Japanese population
	NCT02117648	Recruiting	1	Abemaciclib and clarithromycin or abemaciclib	AST, including breast	No	
	NCT02057133	Recruiting	1	Abemaciclib and Let or abemaciclib and Ana or abemaciclib and Tam or abemaciclib and Exe or abemaciclib and Exe and everolimus	ER$^+$ HER2$^-$ ABC	No	
	NCT02102490	Recruiting	2	Abemaciclib	ER$^+$ HER2$^-$ ABC	No	No more than two lines of CHT allowed (at least 1 for ABC, at least 1 containing taxane)
	NCT02107703	Not yet recruiting	3	Abemaciclib and Ful vs. placebo and Ful	ER$^+$ HER2$^-$ ABC	No	First or second line
	NCT01394016	Active, not recruiting	1	Abemaciclib or abemaciclib and Ful (part G)	AST, including breast (parts D and G)	No	

Migliaccio et al; Curr Opin Oncol 26:568-575,2014

Clinical Development Program
CDK-Inhibitors (Selection)

- NSCLC:
  - KRAS mut: + TKI (erlotinib)
  - KRAS mut: + MEK inhibitors
  - Squamous: monotherapy
- SCCHN: HPV negative only: + cetuximab
  (HPV+: p16 positive)
- Liposarcoma: WDLS/DDLS (CDK amplified in >90%): monotherapy
- Hematologic malignancies: single-agent activity is modest
  + e.g. proteasome inhibitors
- Mantle cell lymphoma: overexpression of cyclin D1: monotherapy
  - BTK resistant: + PI3K inhibitors
  - ≥ 1 prior therapy: + BTK
  - + bortezomib

Clinical Development Program
CDK-Inhibitors (Selection)

- Multiple myeloma: + bortezomib
  + lenalidomid
- Melanoma: + BRAF inhibitors
  + MEK inhibitors
  + PI3K inhibitors
- Rb+ germ cell tumors: monotherapy
  Teratoma
  Teratoma with malignant transformation
  (Non teratomatous germ cell tumor)
- Glioblastoma multiforme: single-agent
- (Esophageal cancer (SCC))
- Pancreatic ductal adenocarcinoma: + TGF beta RK inhibitor
  + MEK inhibitors

The Future in the Development of CDK-Inhibitors

- The cyclin D / CDK4/6 - INK4 - pRb pathway is deregulated in cancer
- Its increased activity results in cell cycle progression
- The cell cycle progression has been shown to be antagonized by small inhibitors of CDK4/6

- Intrinsic activity and potency of different substances of the same class of compounds may differ
- The side-effect profile of different substances may be decisive

The Future in the Development of CDK-Inhibitors

- The development of ideal combinations with inhibitors of the same and/or compensating collateral pathway(s) is key
- The requirement of a combinatorial approach is based on the revelation that no one cyclin or CDK is absolute essential for development
  (Sherr & Robert; Genes Dev 2004)
- The identification and validation of predictive factors for patient selection are of utmost importance