SEASON 2

Understanding Cancer

Lecture 11

Types of signalling pathway: normal and dysregulated BCR-ABL

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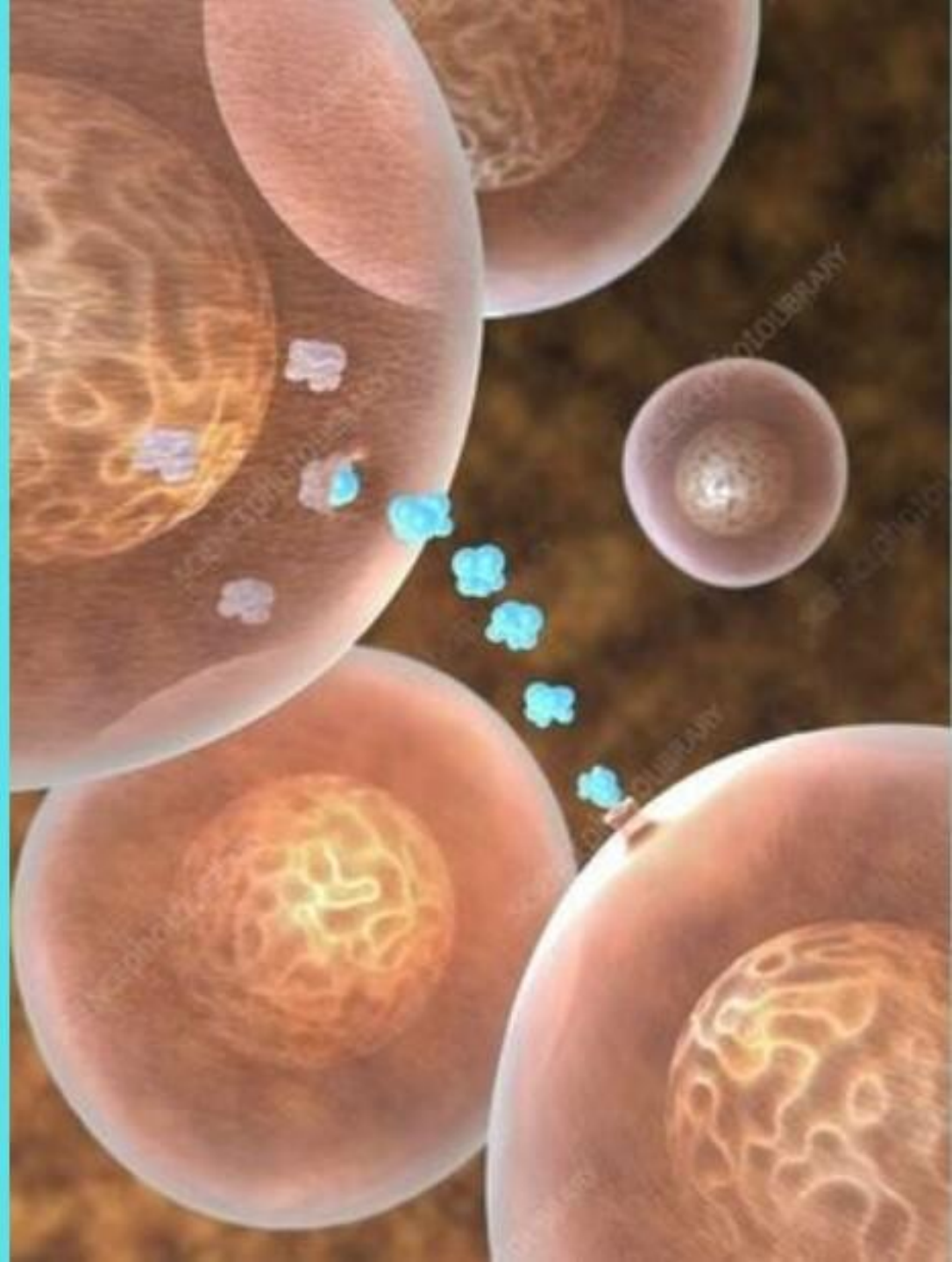

By the end of this lecture, you should understand

- **TGF-β is a pro-inflammatory cytokine that interacts with proteins called SMADs.**
- **SMAD proteins are divided into: regulatory, co-mediators and inhibitory proteins.**
- **TGF-β binds to the C-terminal prodomain latency-related peptide (LAP)** to form **a small latency complex (SLC). It then binds with the TGF-*β* binding protein 1 (LTBP1)** to the **large latent complex (LLC).**
- **The TGF-β ligand must be released from the LLC to make TGF-β ligand active and stimulate SMAD downstream signaling pathway.**
- **TGF-β can promote or inhibit tumour growth.**

What will we learn today?

- ***What is the Philadelphia chromosome?***
- ***The structure of BCR.***
- ***The structure of ABL.***
- ***How is ABL activated?***
- ***Signal Transduction pathways involved with BCR-ABL***
- ***The link between PI3-kinase and BCR-ABL***
- ***Causes of dysregulated pathways***
- ***Chronic myeloid Leukaemia***
- ***B-cell acute lymphoblastic leukaemia (B-ALL).***
- ***Other ABL fusions.***

GENTLE REMINDER
An ideal way of learning:

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

Mini-lectures.

Approximate total time: 1 hour

Divide over 7 days at your **own pace**.

Challenge yourself with a quiz!

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RECAP: How to support your learning?

- **Key facts with diagrams by HN designs presented in a simplified way.**
- **Glossary to help understand what key words mean.**
- **Summary doodle revision posters by HN designs.**
- **Quizzes to test your knowledge and reflect.**
- **Reference list for further reading.**

Acknowledgements: Special thanks to my parents, family, friends and colleagues for their support and the respected teachers and health professions who taught me and installed the passion of cancer/oncology.

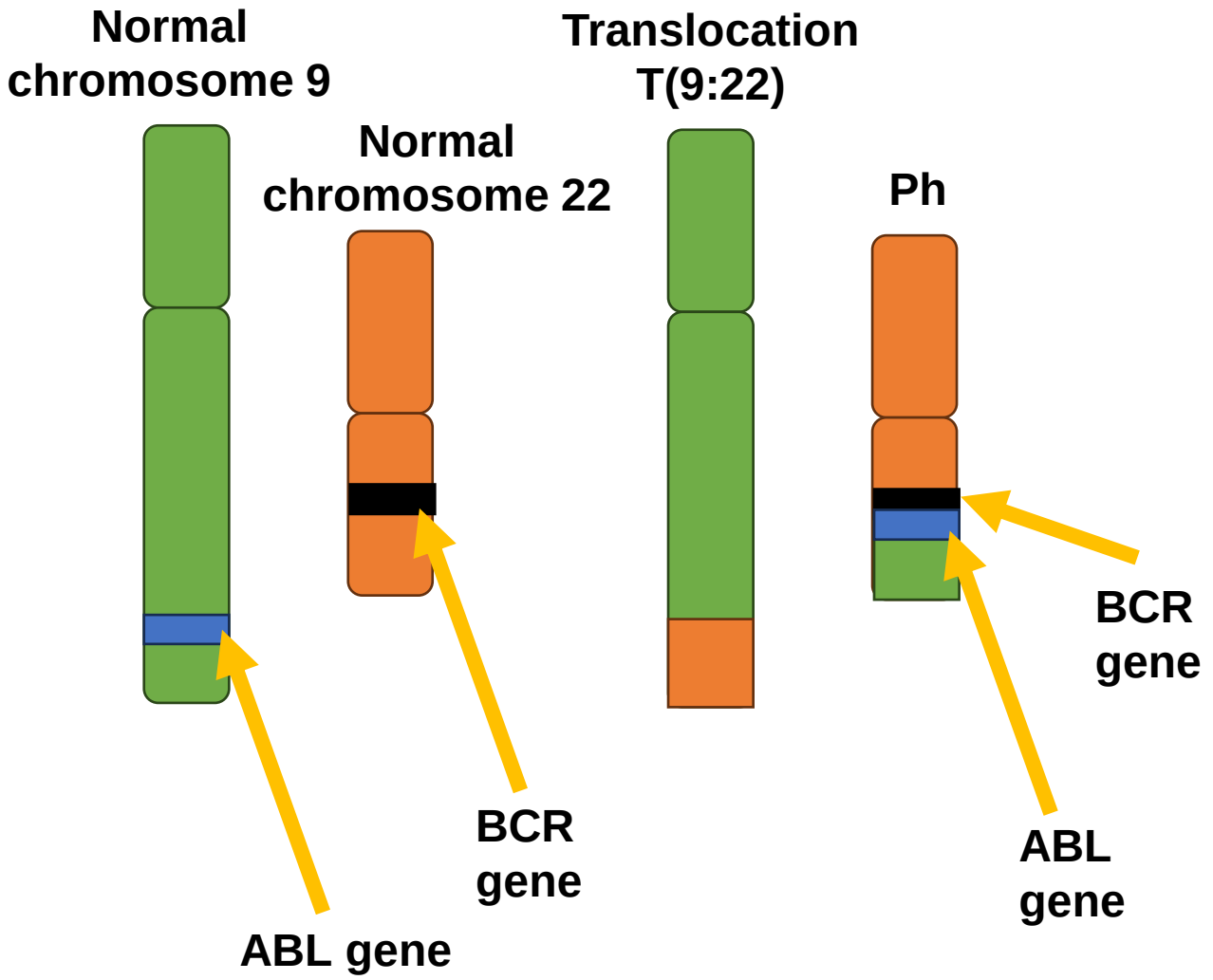
What is the Philadelphia chromosome?

What is the Philadelphia chromosome?

The human Philadelphia (Ph) chromosome arises from a **translocation between chromosomes 9 and 22 t(9;22).**

The fusion occurs between **the ABL1 gene on chromosome 9** and **the breakpoint cluster region (BCR) gene** on **chromosome 22.**

This produces a **tumour-specific fusion chimeric protein called BCR-ABL tyrosine kinase** required for **tumour proliferation, apoptosis and survival.**



(Hantschel, 2012; Li, 2007)

The structure of BCR

The structure of BCR

BCR codes for two proteins: 160kd and 190kd.

Domain	Name	Description	Function
1	Dimerization Motif	**N-terminal coiled-coil motif** It has **63 amino acids** in its sequence. It is common to **other forms of ABL**.	It increases **F-actin binding.** It increases **tyrosine kinase activity of ABL.**
2	Serine/threonine kinase	It contains a **tyrosine amino acid residue 177 (Y177).**	It is a site used for many **adaptor proteins.** It is **phosphorylated by the ABL tyrosine kinase.**
3	GDP-GTP exchange factor (GEF) domain	It is the **central point.** It contains **DBL protooncogene-like sequences of GEF.** They regulate **growth and differentiation**.	DBL catalyses **the exchange of guanine nucleotides** on **the RAS-related molecule Cdc42Hs.**
4	RAC-GAP	It contains **homologous sequences to the catalytic domains of GTPase activating proteins (GAP).** GAP are part of the **GTP protein binding family p21rho and p21 rac.**	It controls **the rate of GTP hydrolysis** of **RAS proteins to their inactive forms.** This facilitated **downstream signalling.**

(Alpf medical, 2018; Hantschel, 2012)

The structure of ABL

The structure of ABL

- ABL encodes a **145-kd protein (p145ABL).**
- It is a **non-receptor tyrosine phosphokinase** located in the **cytoplasm.**
- There are **low kinase activity of the full-length Abl protein *in vitro*** and **hard to detect in unstimulated cells.**
- It has two members: **Abl and Arg (Abl-related gene)** encoded by the **ABL1 and ABL2 genes in humans.**

(Alpf medical, 2018; Hantschel, 2012; Jing *et al*., 2017)

The structure of ABL

Domain	Name	Function
1	Myristoylation	It adds the 14-carbon fatty acid, myristate, to the N-terminal glycine residue of a protein via a covalent bond. It is a lipid modification step. It is rarely attached to a lysine residue.
SH3	SRC homology (SH) kinase	It negatively regulates the tyrosine kinase activity of ABL where it can interact with inhibitors. Autoinhibitory affect.
SH2	SRC homology (SH) kinase	It regulates the tyrosine kinase function of ABL. It can attach to tyrosine phosphorylated proteins via arginine residues.
SH1	SRC homology (SH) kinase	Catalytic domain. Tyrosine kinase domain
2	Catalytic Domain	
3	Binding site	It has DNA-binding domain, nuclear localization signals, and a binding site for actin. It facilitates protein to protein interactions

(Alpf medical, 2018; Hantschel, 2012; Jing *et al*., 2017)

How is ABL activated?

How is ABL activated?

Method 1

- **By itself via point mutations and deletions.**

Method 2

- **Fusion of GAG.**
- **The binding of SH3 and SH2 domains to their respective ligands**

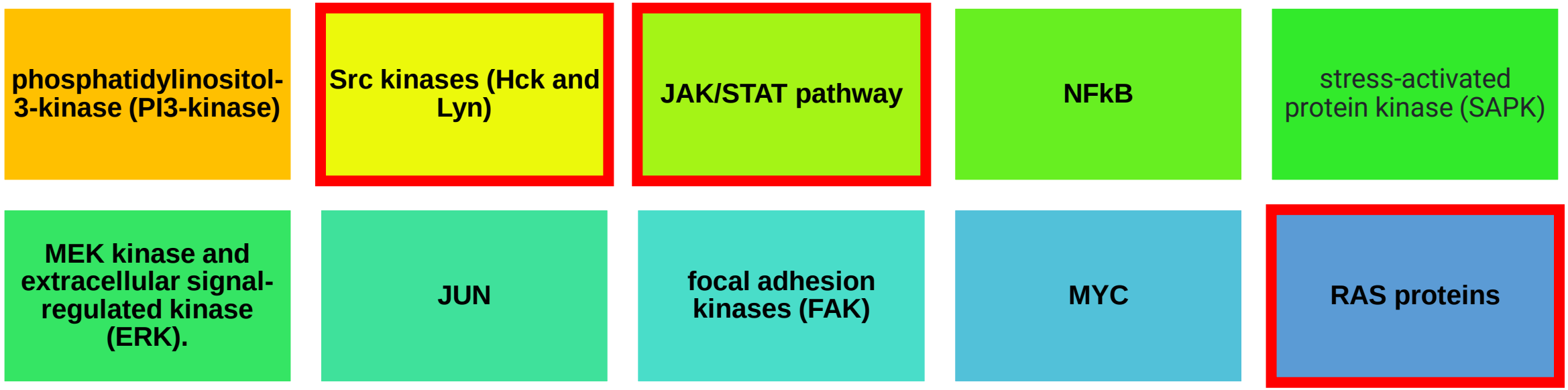
Signal Transduction pathways involved with BCR-ABL

Signal Transduction pathways involved with BCR-ABL

phosphatidylinositol-3-kinase (PI3-kinase)	Src kinases (Hck and Lyn)	JAK/STAT pathway	NFkB	stress-activated protein kinase (SAPK)
MEK kinase and extracellular signal-regulated kinase (ERK).	JUN kinase (JNK) pathway	focal adhesion kinases (FAK)	MYC	RAS proteins

(Alpf medical, 2018; Li, 2007)

Signal Transduction pathways involved with BCR-ABL



(Alpf medical, 2018; Li, 2007)

Src kinase

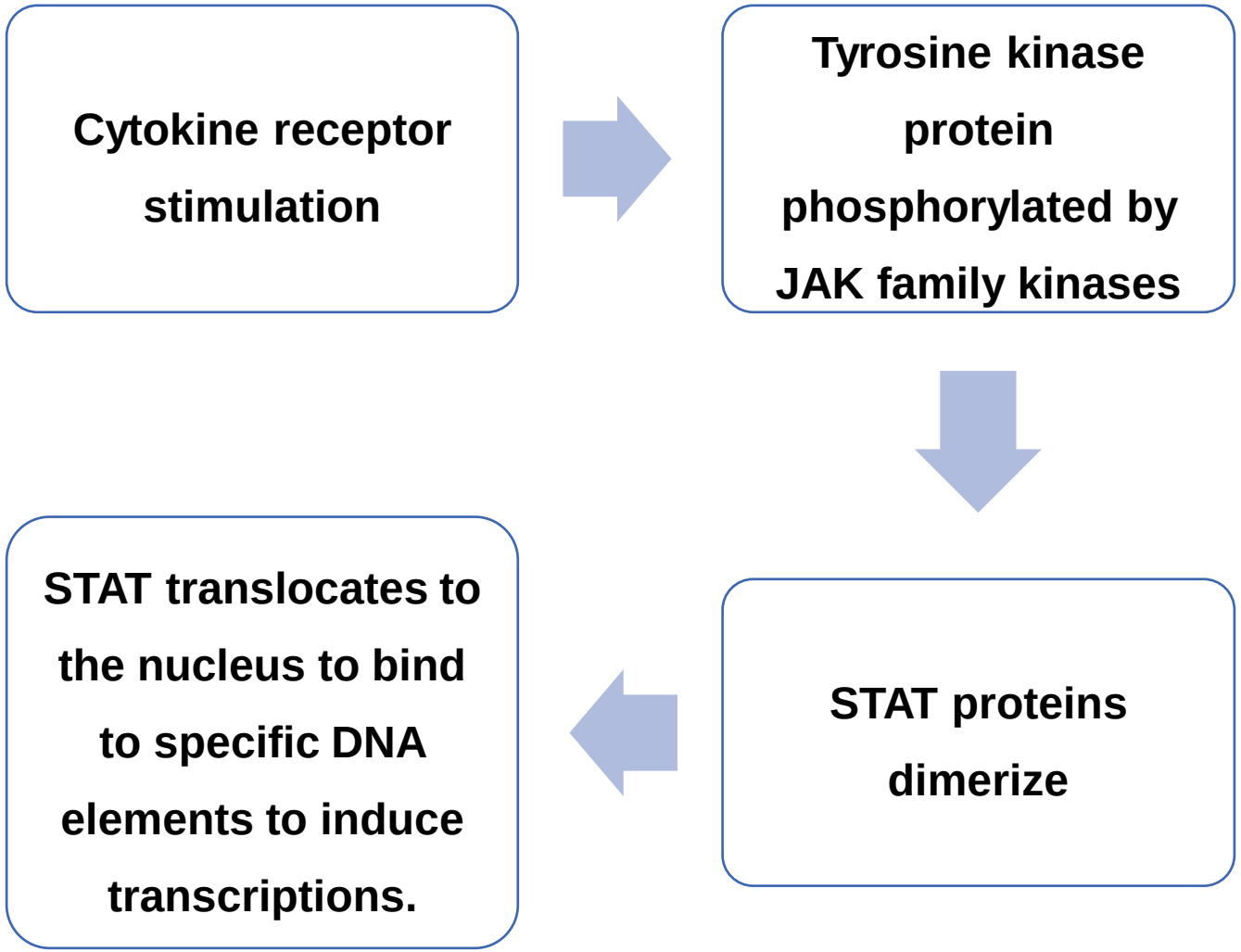
- Src family kinases are **structurally related non-receptor protein tyrosine kinases.**
- They have **four SH domains.**
- There are *ca*. eight family members: **Blk, Fgr, Fyn, Hck, Lck, Lyn, c-Src, and Yes.**
- **BCR-ABL can activate Lyn and Hck in myeloid cell lines.**
- **Src kinases** may be **linked indirectly to BCR-ABL** through **other signaling molecules e.g. protein tyrosine phosphatases.**
- **Activation of Src kinases** is **NOT DEPENDENT on BCR-ABL**.

(Warmuth *et al*., 2003; Lowell and Soriano, 1996; Li, 2007)

STAT5/JAK2

STAT proteins (signal transducers and activators of transcription) associate the **cytokine receptor stimulation** to **induce transcription.**

STATs are present in **Bcr-Abl–expressing cells (pi90BCR-ABL and p210BCR-ABL)** to induce **leukaemia (leukemogenesis)** especially **STAT1, STAT5 and STAT6.**



(Alpf medical, 2018; Li, 2007)

RAS proteins are important G-proteins in **signal transduction, proliferation, and malignant transformation**.

In the active state, **RAS proteins transduce the signals that are regulated by:**

GTPase-activating proteins (GAPs)

- **GAPs enhance the rate of GTP hydrolysis to GDP.**
- **Negatively regulate RAS function.**

guanine nucleotide exchange factors (GEFs)

- **GEFs induce exchange of GDP for GTP on RAS.**
- **Positively regulate RAS function.**

(Alpf medical, 2018; Li, 2007)

How does RAS get activated via BCR-ABL?

GRB2 linked with its SH2 domain to the conserved tyrosine residue Y177 within BCR.

This binding recruits adapter protein, SOS ("son-of-sevenless"), which stimulates RAS.

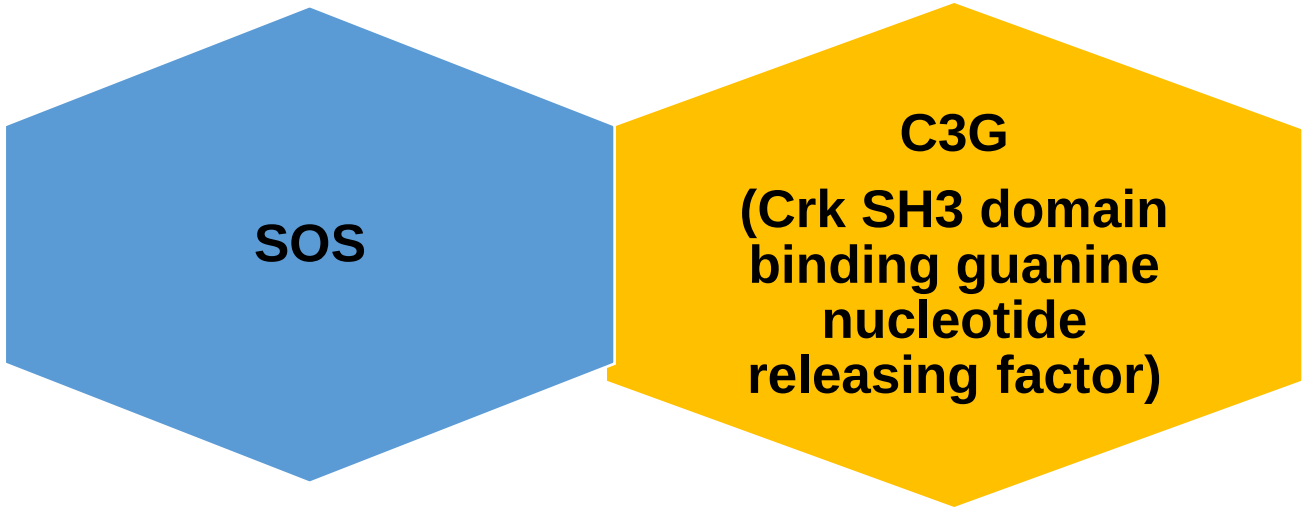
RAS can be also activated by inhibiting GAP.

RAS can be also activated by CRKL via SOS.

(Alpf medical, 2018; Li, 2007)

CRKL

- An **adaptor protein that creates a link between p210BCR-ABL** and **focal adhesion molecules: FAK, actin, and paxillin.**
- It can be **phosphorylated via its tyrosine kinase domain** in cells that contain the **Philadelphia chromosome.**
- It binds to **proline amino acid residues in BCR-ABL** and can stimulate RAS by:



(Alpf medical, 2018; Li, 2007; Shakyawar *et al.* 2018)

The link between PI3-kinase and BCR-ABL

The link between PI3-kinase and BCR-ABL

- It is composed of:

1) A p85 regulatory subunit.

2) A p110 catalytic domain via its enzymatic activity.

- PI3K is possibly associated with **BCR-ABL via CBL (casitas B-lineage lymphoma protein).**
- CBL is a **proto-oncogene** that is **phosphorylated in BCR-ABL expressing cells** via its **tyrosine residues.**
- BCR-ABL associated with **apoptosis:**

1. **Promoting apoptosis via BCL-2 and BCL-XL.**
2. **Evading apoptosis in chronic myeloid leukaemia (CML) by downregulating cyclin-dependent kinase inhibitor p27.**

Causes of dysregulated cancer pathways

Causes of dysregulated cancer pathways

Direct interaction with retinoblastoma by overexpression of ABL.

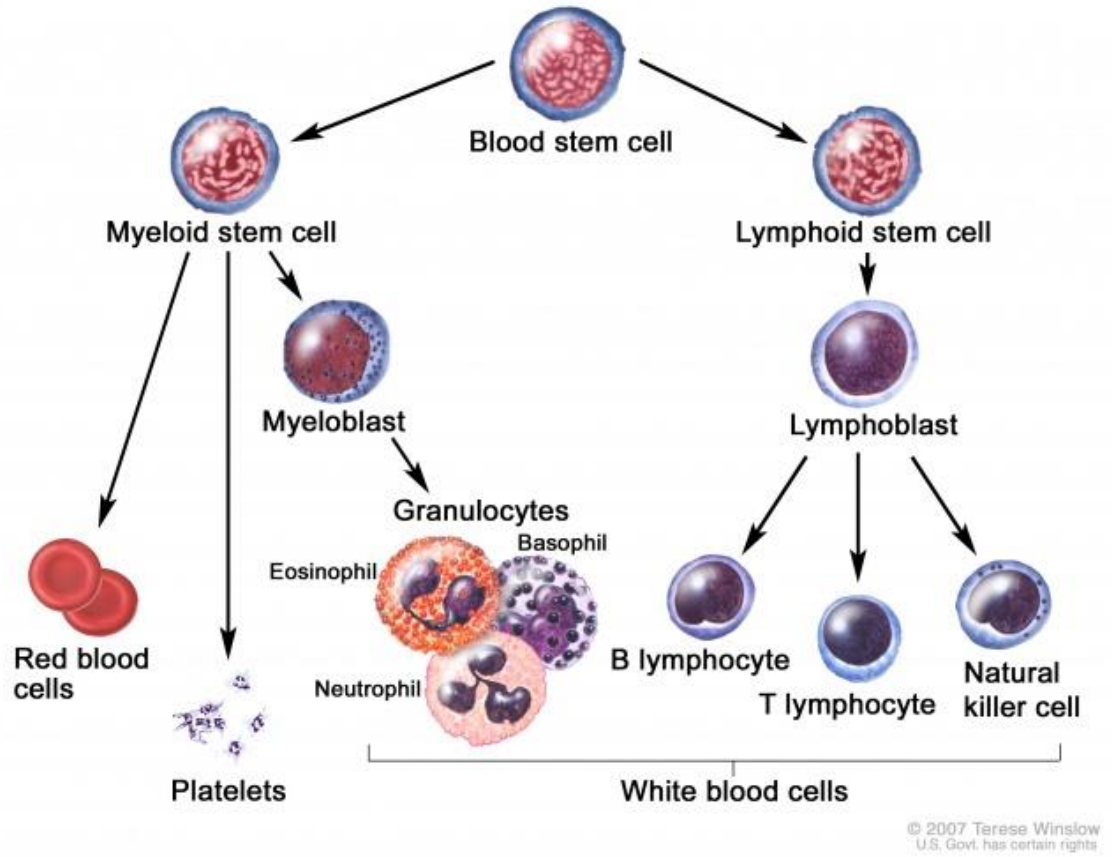
Defects in SH2 domain which affects protein to protein interactions and lowers phosphotyrosine binding.

Direct interaction with p53 by overexpression of ABL.

Chronic myeloid leukaemia

Chronic myeloid leukaemia (CML)

- **CML is a cancer that affects the myeloid the white blood cell precursors (myeloblasts) in the bone marrow that form a type of white blood cell called granulocytes.**
- **CML begins with a chronic phase** and can **progress to a terminal blastic phase.**
- The **BCR-ABL tyrosine kinase inhibitor imatinib mesylate (Gleevec) partially eliminate BCR-ABL-expressing leukemic cells** and **patients can develop drug resistance.**
- **Imatinib-inhibited BCR-ABL** can still **stimulate Src kinases, which can lead to acute lymphoblastic leukaemia (ALL).**



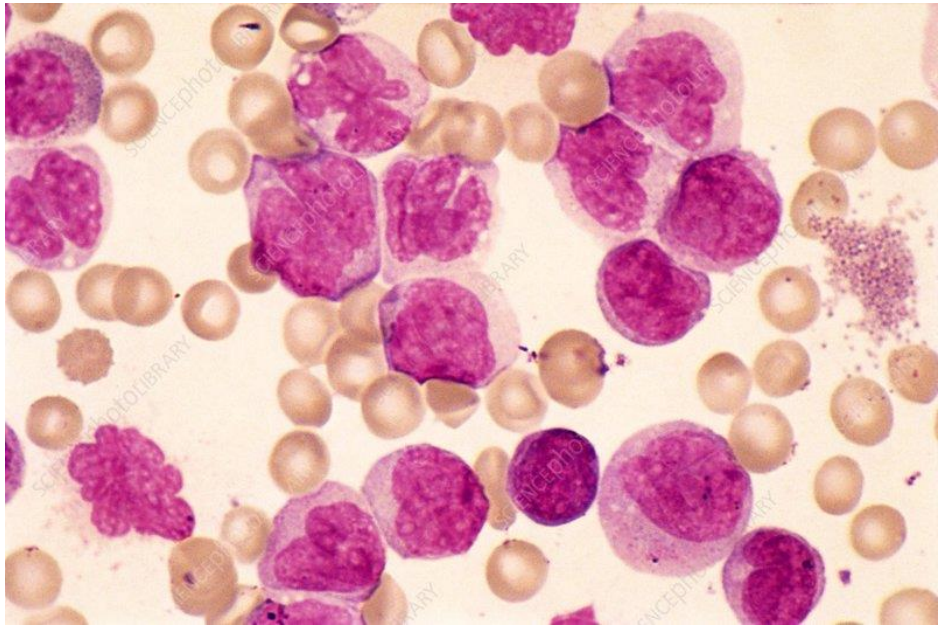
(Graham *et al*. 2002)

Chronic myeloid leukaemia (CML)

It affects people of middle age of any genders and between 40-60 years of age.

Key symptoms

- Fever
- Weight loss
- Sweat
- anaemia
- Blurred vision



Light micrograph of blood cells from bone marrow in a CML patient.
Pink cells →white blood cell precursors (myeloblasts) or white blood cells.
Orange ones → red bloods cells.
Magnification: x1000
(Secchi-Lecaque and Roussel-Uclaf, 2023)

p62dok

- CML patients have **BCR-ABL adaptor protein called p62dok**.
- It associates with **RAS GAP which inhibits its phosphorylative activity.**
- It forms **complexes with the SH2 domain-containing phosphatidylinositol polyphosphate 5-phosphatase (SHIP1)** in **hematopoietic cells expressing BCR-ABL.**

(Alpf medical, 2018; Li, 2007)

B-cell acute lymphoblastic leukaemia (B-ALL).

B-cell acute lymphoblastic leukaemia (B-ALL).

Abnormal cell → lymphoblast in the blood

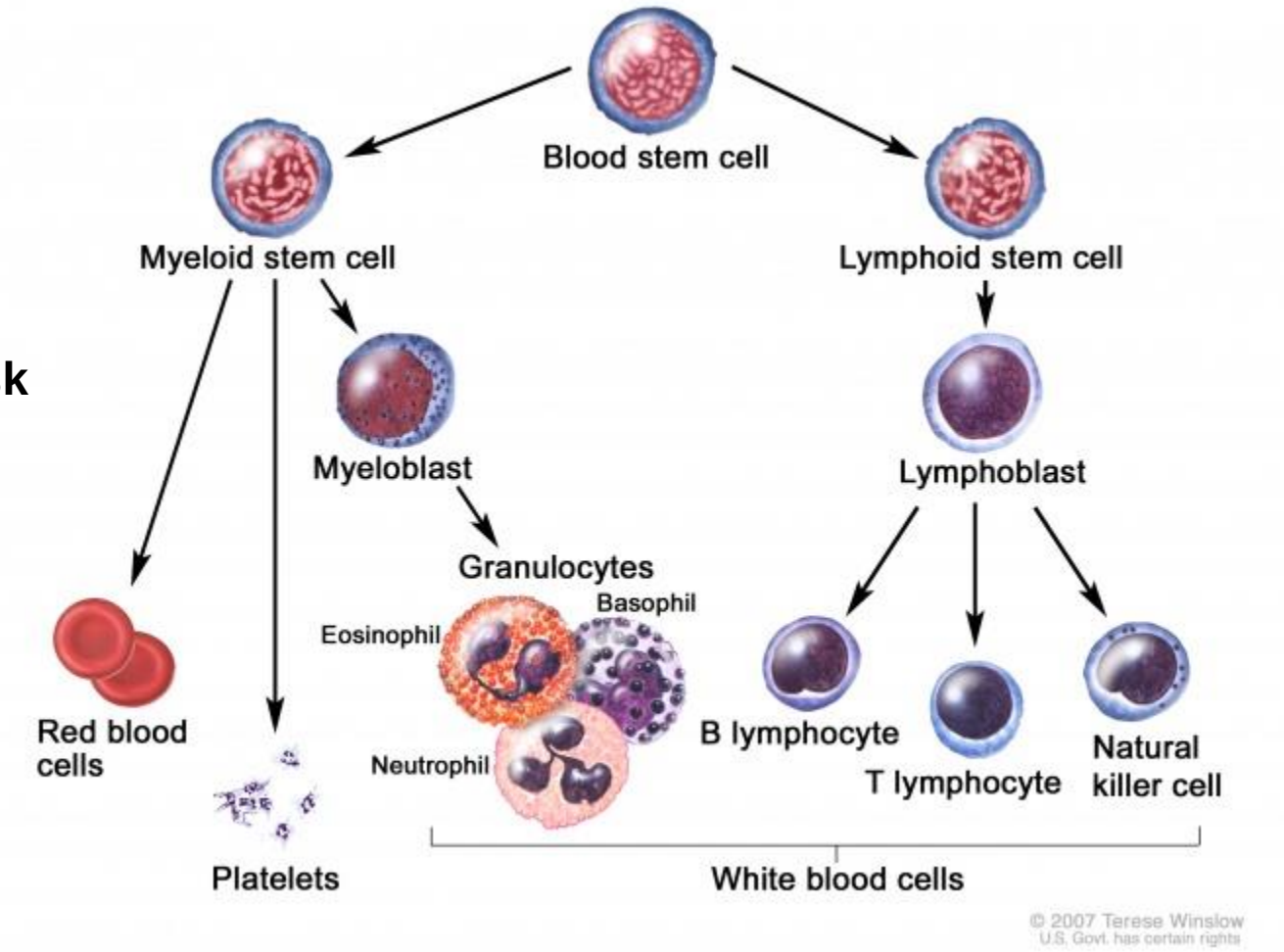
Children under aged 15 years

Cause unknown but associated with **increased risk**

with inherited or genetic conditions

Symptoms

- **Anaemia**
- **Bleeding**
- **infection**
- **Hepatosplenomegaly**



Other Abl fusions

Other Abl fusions - Nup214-Abl

- 7% of cases with **T-cell acute lymphoblastic leukemia (T-ALL) with long latency that Bcr-Abl myeloid leukaemia.**
- The **first exon of ABL1 is missing in the Nup214-Abl fusion protein.**
- It is caused by **extra-chromosomal amplification of a ~500-kb region of the long arm of chromosome 9.**
- This fuses with **most of NUP214 exons to ABL1.**
- They vary in **efficacy to Bcr-Abl tyrosine kinase inhibitors.**

By the end of this lecture, you should understand

- **The human Philadelphia (Ph) chromosome arises from a translocation between chromosomes 9 and 22 of the t(9;22). This affects the ABL and BCR genes.**
- **There are two main cancers associated with ABL-BCR translocation: chronic myeloid leukaemia (CML) that mainly affect middle-aged patients and B-cell acute lymphoblastic leukaemia (B-ALL) that affect paediatric patients under 15 years old**
- **Other Abl fusion can occur that can cause T-cell acute lymphoblastic leukemia (T-ALL).**
- **RAS is the main signal transduction pathway linked by BCR-ABL. Upon activation of BCR with GRB2, it recruits SOS ("son-of-sevenless") to stimulate RAS.**
- **RAS can be also activated by inhibiting GAP or activated by CRKL via SOS.**

Reference list for further reading

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SEASON 2

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Understanding Cancer

Lecture 12

Types of signalling pathway: normal and dysregulated Notch

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