lymphocyte seeing

Lymphocyte seeing

CHAPTER 1

DISCOVERING CLL
CHRONIC LYMPHOCYTIC LEUKEMIA

You see...

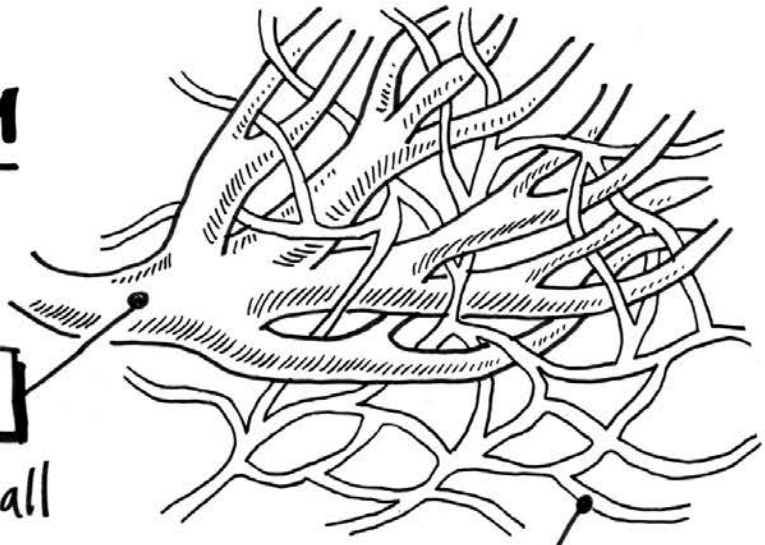
The Lymph nodes are linked to the LYMPHATIC SYSTEM that runs through your body...

There are around 800 of them, located mostly in the areas we palpated.

I also palpated your liver and spleen as they are linked to this system...

LYMPHATIC AND VASCULAR SYSTEM

Let's take a closer look:



THE VASCULAR SYSTEM

Allows blood to flow to all of our organs.

It hosts:

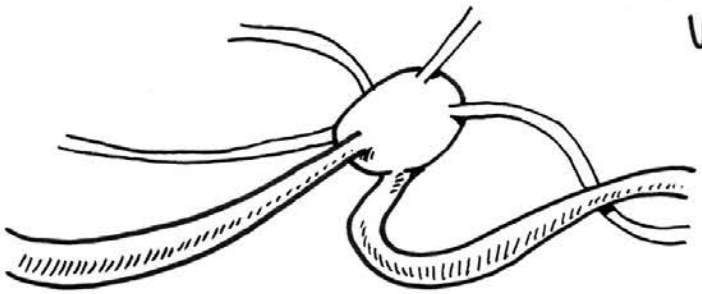
- **RED BLOOD CELLS** to deliver oxygen
- **PLATELETS** for coagulation
- **WHITE BLOOD CELLS** including **LYMPHOCYTES**, part of the body's immune system.

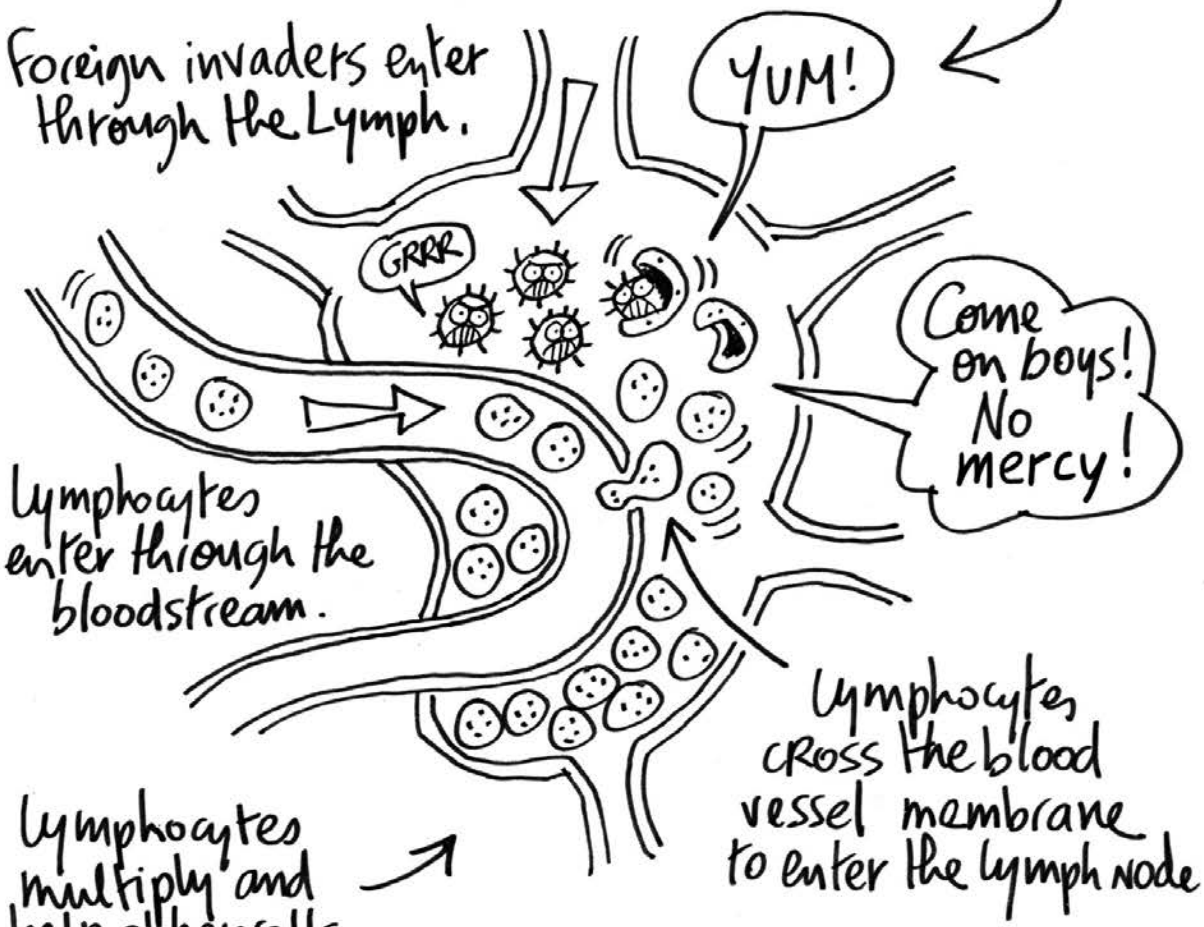
THE LYMPHATIC SYSTEM

Where, parallel to THE VASCULAR SYSTEM, a liquid called **LYMPH** circulates, draining and cleaning the whole body.

THE SENTINELS

THE LYMPH NODES are small organs located on the LYMPHATIC SYSTEM where the cells of the immune system will encounter and eliminate foreign particles.





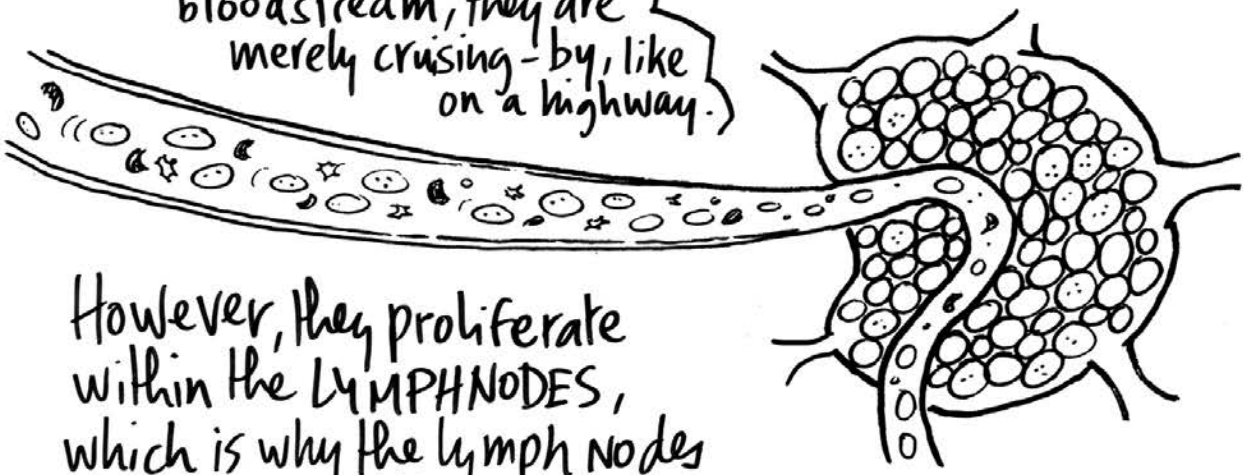
That's why in the case of a local infection the nearest lymph node will inflate, and then deflate once the infection is halted. In a healthy body, lymph nodes are present but are too small to be palpable.

CLL

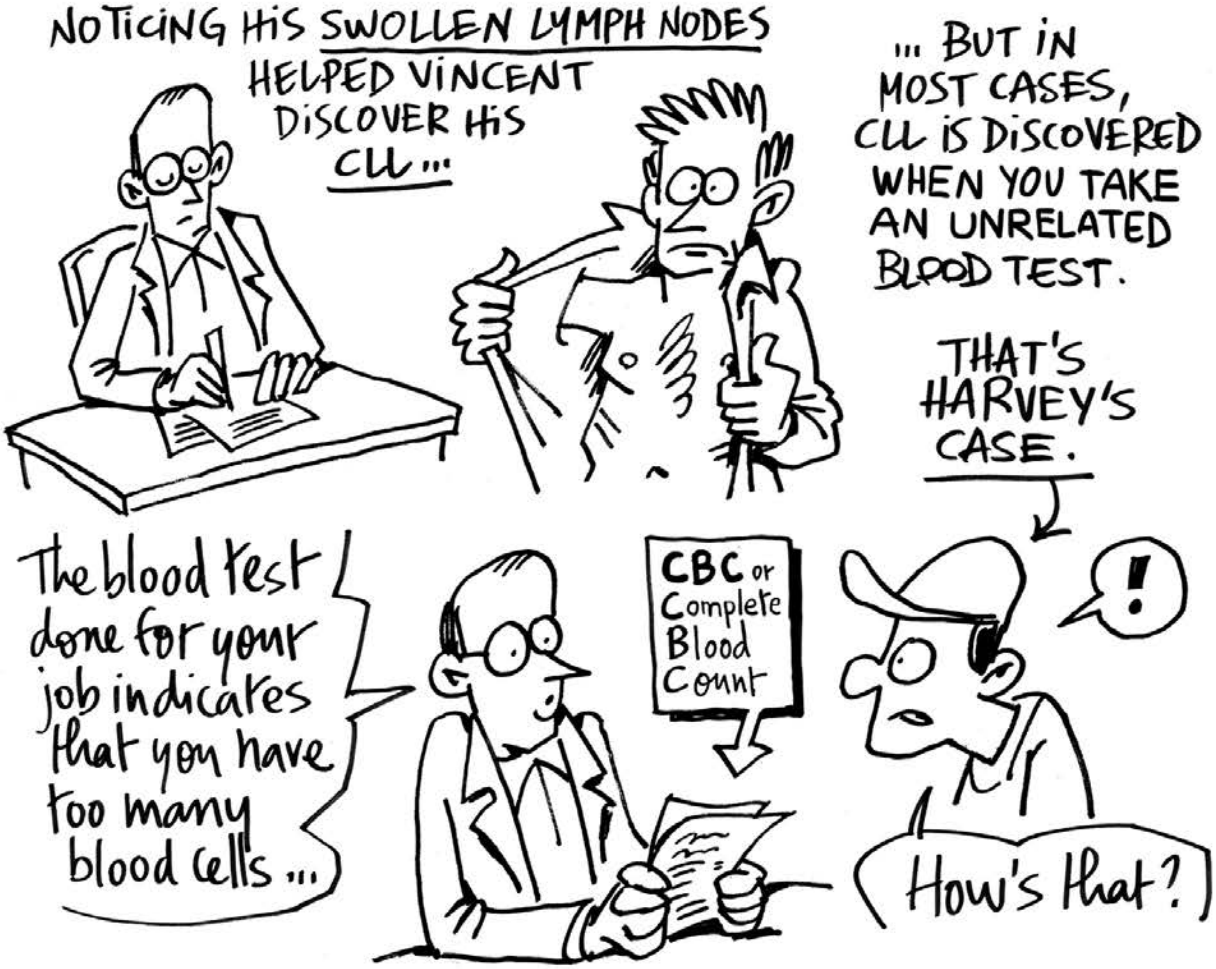
Our body produces a sufficient amount of cells to replace the ones that die. In the case of CLL, not enough lymphocytes die, and new ones keep being produced, so there is ACCUMULATION.

That increase of their number in the bloodstream is called LEUKEMIA.

Lymphocytes don't multiply within the bloodstream, they are merely cruising-by, like on a highway.



However, they proliferate within the LYMPHNODES, which is why the lymph nodes inflate when the disease is acute.
When the lymph nodes grow too much in size, treatment becomes necessary. Treatment isn't urgent in most cases. It is during monitoring that the decision to treat may be taken!



WHITE BLOOD CELLS ARE MADE OF:

NEUTROPHILS

They are the "hungry enzymes" of the body: They swallow any pathogen they encounter.

LYMPHOCYTES

They destroy cells by producing antibodies or by releasing molecules.

WE WILL ALSO FIND IN SMALLER NUMBERS:

EOSINOPHILS

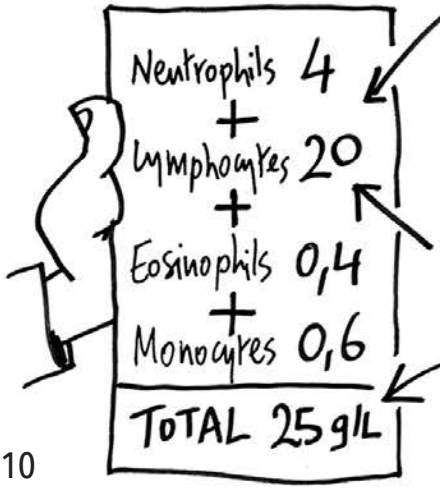
As hungry as the Neutrophils, but hungry for parasites.

MONOCYTES

They help to clean up the destroyed cells.

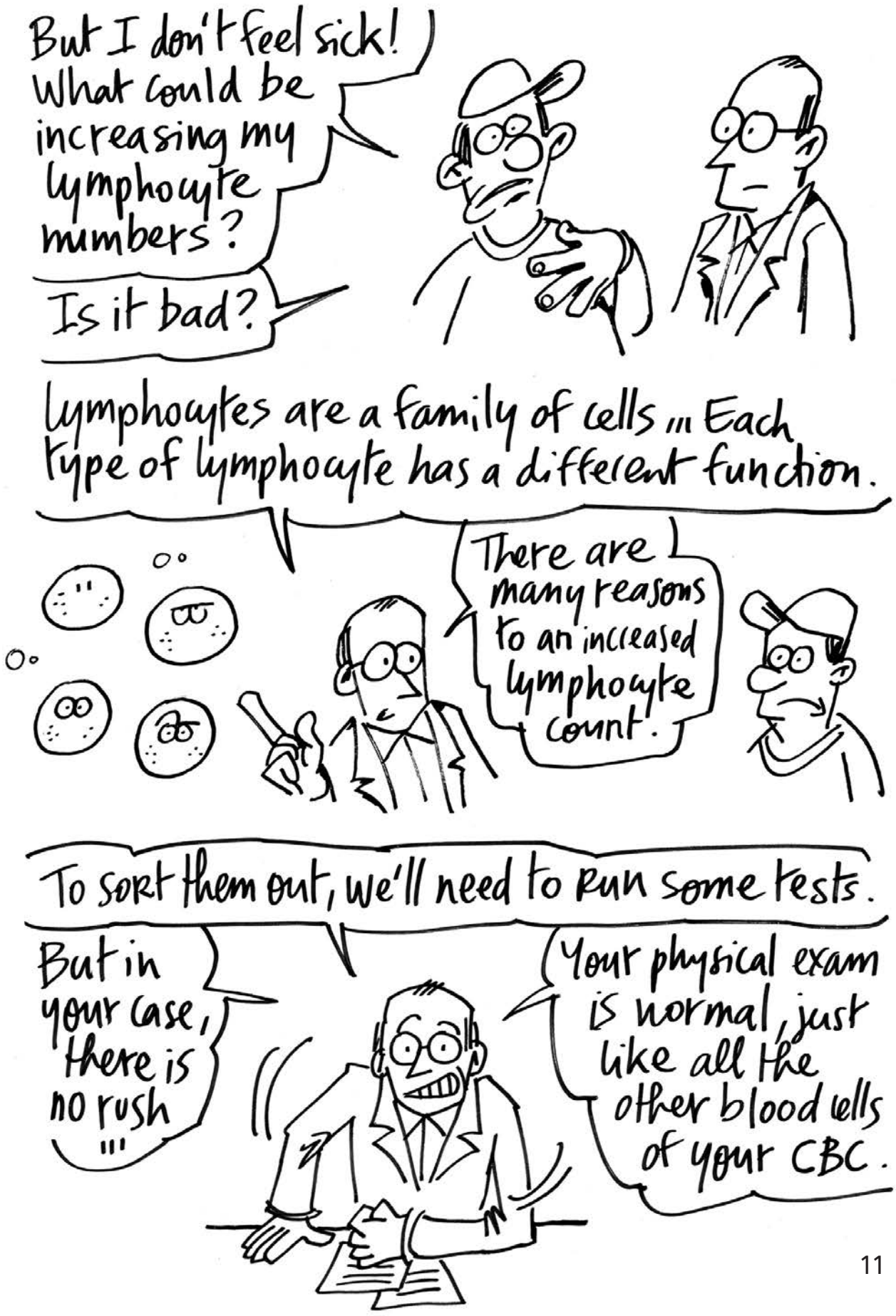
With CLL, the CBC will usually show an increased count of lymphocytes in the bloodstream as the sole anomaly.

We call it "lymphocytosis" when the count is higher than 4000/mm³ (or 4g/L). With CLL it's often much more.



As white blood cells are made of the sum of its different kinds, when the lymphocyte count is high, so is the white blood cell count.

≠ instead of <10 g/L

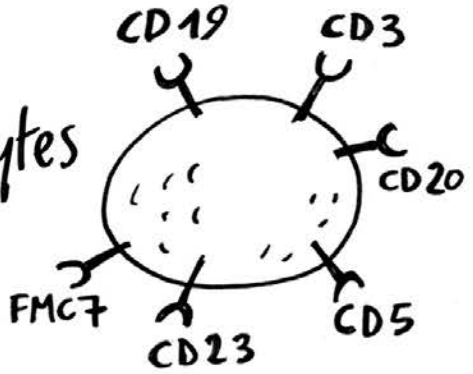

But I don't feel sick! What could be increasing my lymphocyte numbers?
Is it bad?
Lymphocytes are a family of cells... Each type of lymphocyte has a different function.
There are many reasons to an increased lymphocyte count.
To sort them out, we'll need to run some tests.
But in your case, there is no rush...
Your physical exam is normal, just like all the other blood cells of your CBC.

CHAPTER 2

DIAGNOSIS

For both Vincent who has swollen nodes and Harvey who has an increased lymphocyte blood count, an exam is a must !!!

IMMUNOPHENOTYPING OF THE PERIPHERAL BLOOD. The identification of different types of lymphocytes is made possible by the analysis of their various surface antigens...

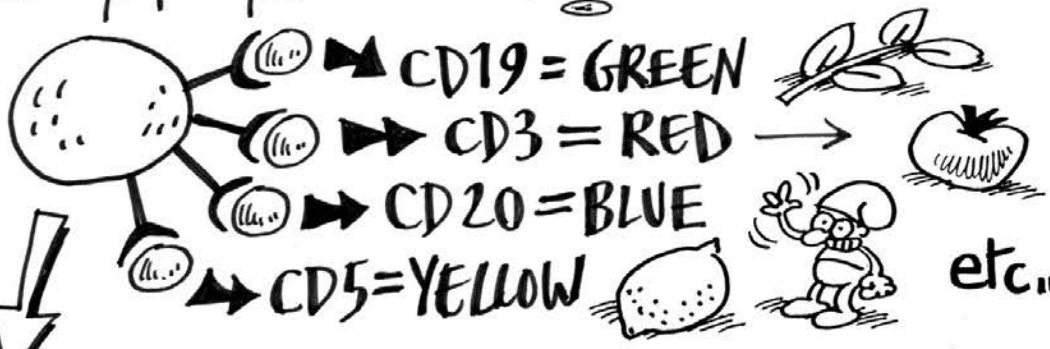


#1 A blood sample is taken

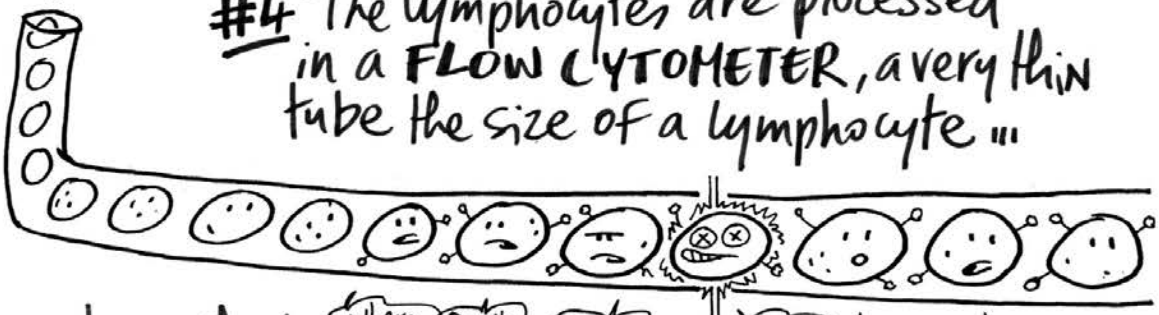
then placed in a centrifuge.

#2 The white blood cells are isolated then mixed with FLUOROPHORE marked antibodies.

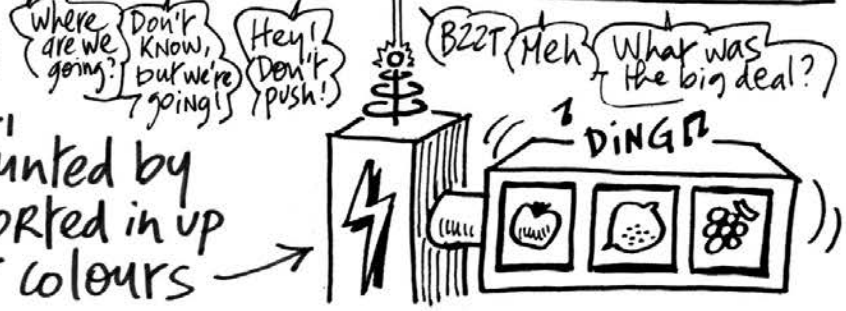
#3 FLUOROPHORE is a fluorescent compound that will stain the antibodies. They will attach to surface antigens and color each lymphocyte differently.



#4 The lymphocytes are processed in a **FLOW CYTOMETER**, a very thin tube the size of a lymphocyte...

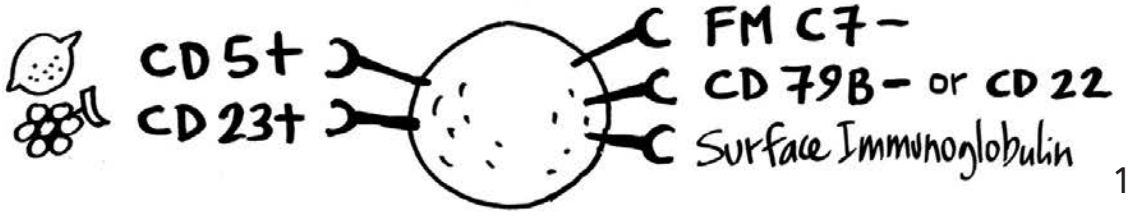


...where, thanks to Fluorescence, each cell is counted by a laser, and sorted in up to 10 different colours →



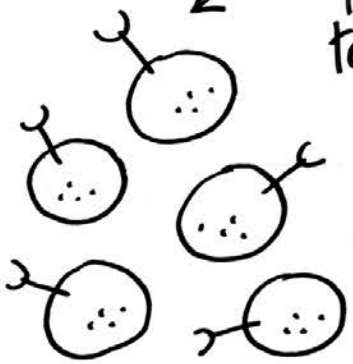
The presence or absence of 5 specific markers will establish the presence or absence of sick cells.

THESE MARKERS ARE IN EXCESS. OR **THESE MARKERS WERE LOST**




5/5
That's how we get the "MATUTES SCORE". A result greater than or equal to 4 meaning CLL.
For proper diagnosis, a Bone Marrow BIOPSY (bone marrow sample drained out from a sternum puncture) is no help for CLL. Immunophenotyping of the peripherical blood is the key exam.
Lastly, this exam allows an eshimate of CLL abnormal lymphocytes in the bloodstream when their amount is greater than 5000/mm3 and they show both markers CD19+CD5.
All those data are key to establish a precise diagnosis and an efficient monitoring of the disease.
You both have a 5/5 MATUTES SCORE, in other words, you have CLL.
5/5

During the immunophenotyping, we can add other markers which will tell us more about the cell's activity and their ability to multiply. Such is the case of the CD 38 marker.

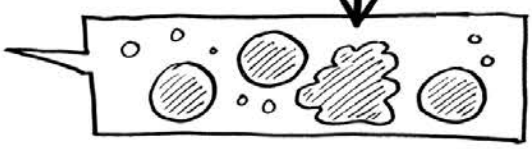


In Vincent's lymph nodes, CD38 is expressed by the cells.

Harvey's cells do not express it.

Are we able to identify CLL lymphocytes on the CBC?

We aren't, because whatever their function is, and whether they're normal or leukemic, lymphocytes all look the same under the microscope... We can only spot GÜMPRECHT SHADOWS...



...which are mangled cells, since their membrane is more fragile than normal lymphocytes' membranes. But that doesn't allow for a definite diagnosis, nor does it have any unfavorable signification.

So what can we do Now?

(Am I gonna need more tests?

(Am I gonna need treatment?

CHAPTER 3

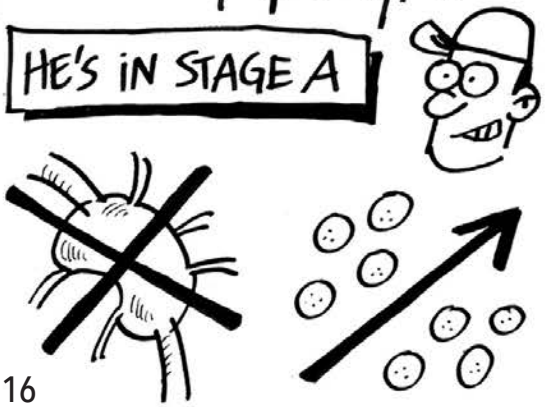
INITIAL WORK-UP

In the 80's, Prof. Binet classified CLL in 3 stages: A, B & C. Back then, there were very few biological tests that could predict if the sickness was going to evolve or not. That was all we had to make the decision to start treating or not.

Nowadays, specialised biological tests help us modulate this decision. Staging is based on clinical exams (lymph node, liver and spleen palpation) as well as the CBC.

Harvey's lymph nodes aren't swollen and his CBC shows only an increased level of lymphocytes.

HE'S IN STAGE A

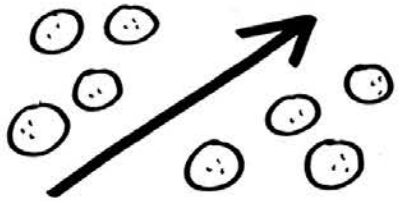


Vincent lymph nodes are swollen, in his neck, under his arms and in his groin.

Those are the 3 major lymph node clusters.

The liver and the spleen are also checked for any swelling. After a deep breath the doctor also palpated Vincent's spleen tip. That he was able to palpate the spleen means the spleen has inflated even if Vincent doesn't feel it.

His CBC shows only an increased number of lymphocytes, with no further anomalies



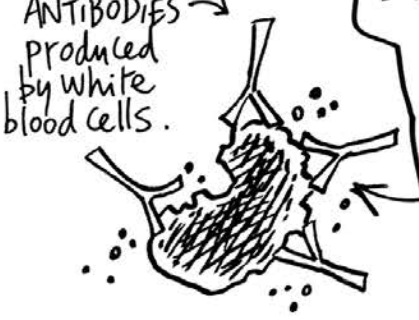
VINCENT IS IN STAGE B

STAGE C is characterised by lower red blood cells and/or platelet counts.

They are called **CYTOPENIAS**.

You see, the various blood cells (red blood cells, white blood cells and platelets) are produced in the BONE MARROW: it's the manufacturing plant.

Occasionally lymphocytes will proliferate like weeds in a garden (which is what happens with CLL and Waldenström's Macroglobulinemia), suffocating red blood cells and platelets. In other cases, cells are being produced normally, but are getting destroyed by antibodies. When this happens to Red Blood Cells, it's called HEMOLYTIC ANEMIA.



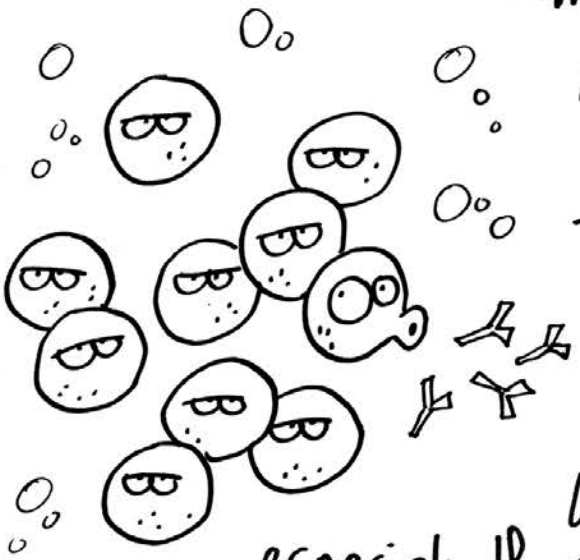

So if I understand correctly, my bone marrow, my "factory", can sometimes get jammed?
Yes, That's it...
... And other times, there can be antibodies destroying my red blood cells and my platelets...
Absolutely! But for now, you don't need treatment and you might never need any. In fact, as long as CLL is in STAGE A, it will not assault your body. It can stay in STAGE A indefinitely, in which case less than half of the patients will ever need CLL treatment.
As for you Vincent, you need treatment, but it's not urgent. To establish which one you need, we'll need to conduct more tests.

CHAPTER 4

MONITORING

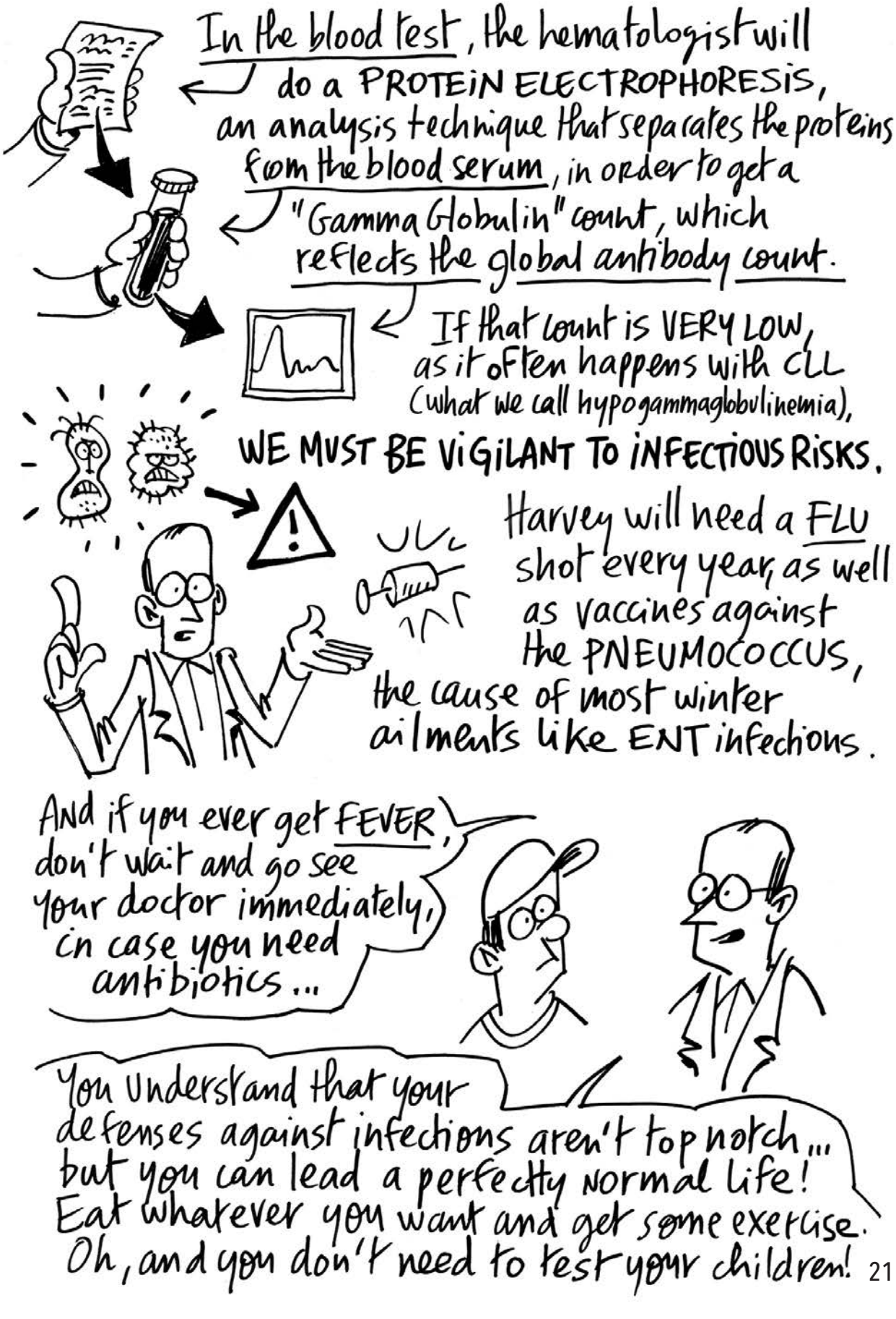
HARVEY LEAVES HIS APPOINTMENT WITH NO PRESCRIPTION BUT A LOT OF QUESTIONS...

He will see his hematologist every 6 months.



Monitoring focuses mainly on <u>infections</u>.

That's because CLL increases the leukemic lymphocytes numbers but decreases the normal lymphocytes numbers, especialy those that produce the <u>ANTIBODIES</u> necessary to fight against germs.

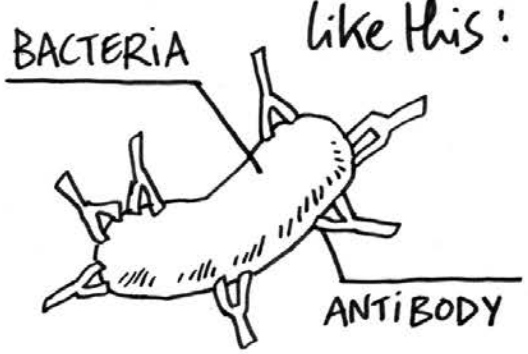

In the blood test, the hematologist will do a PROTEIN ELECTROPHORESIS, an analysis technique that separates the proteins from the blood serum, in order to get a "Gamma Globulin" count, which reflects the global antibody count.
If that count is VERY LOW, as it often happens with CLL (what we call hypogammaglobulinemia),
WE MUST BE VIGILANT TO INFECTIOUS RISKS.
Harvey will need a FLU shot every year, as well as vaccines against the PNEUMOCOCCUS, the cause of most winter ailments like ENT infections.
AND if you ever get FEVER, don't wait and go see your doctor immediately, in case you need antibiotics...
You understand that your defenses against infections aren't top notch... but you can lead a perfectly normal life! Eat whatever you want and get some exercise. Oh, and you don't need to test your children!

CHAPTER 5

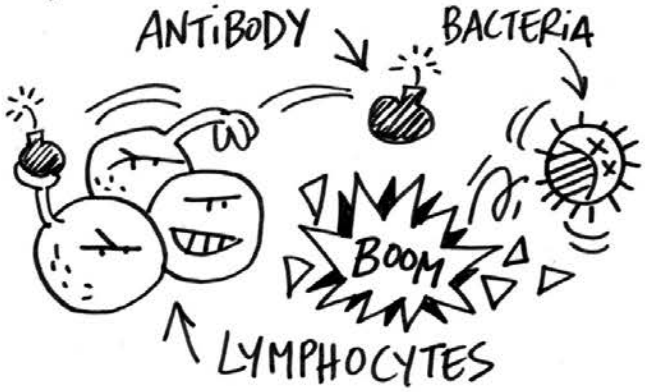
WALDENSTRÖM'S MACROGLOBULINEMIA

IN THE DOCTOR'S WAITING ROOM, HARVEY MEETS GEORGE. THEY START COMPARING THEIR ELECTROPHORESIS CHARTS.

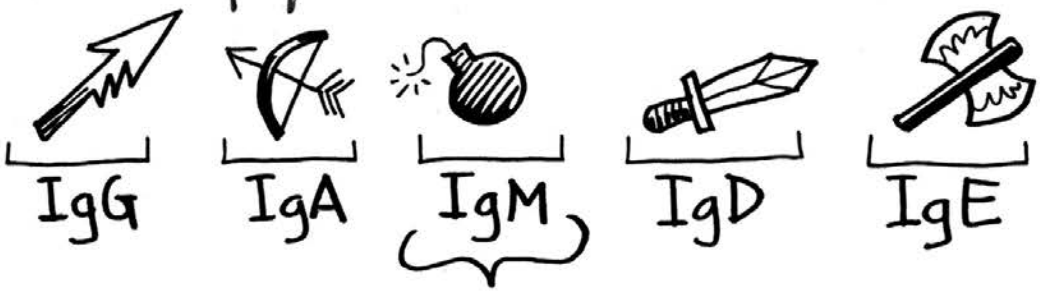
From a medical point of view, it looks a little like this:



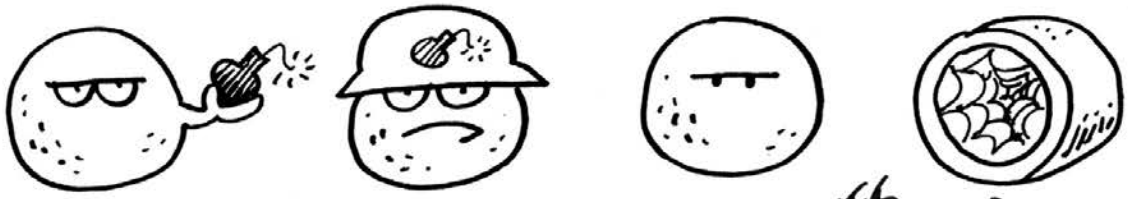
But we can also picture it like this:



There are many types of antibodies, all suited to the many types of bacterias and viruses.



IMMUNOGLOBULIN M is produced by ...

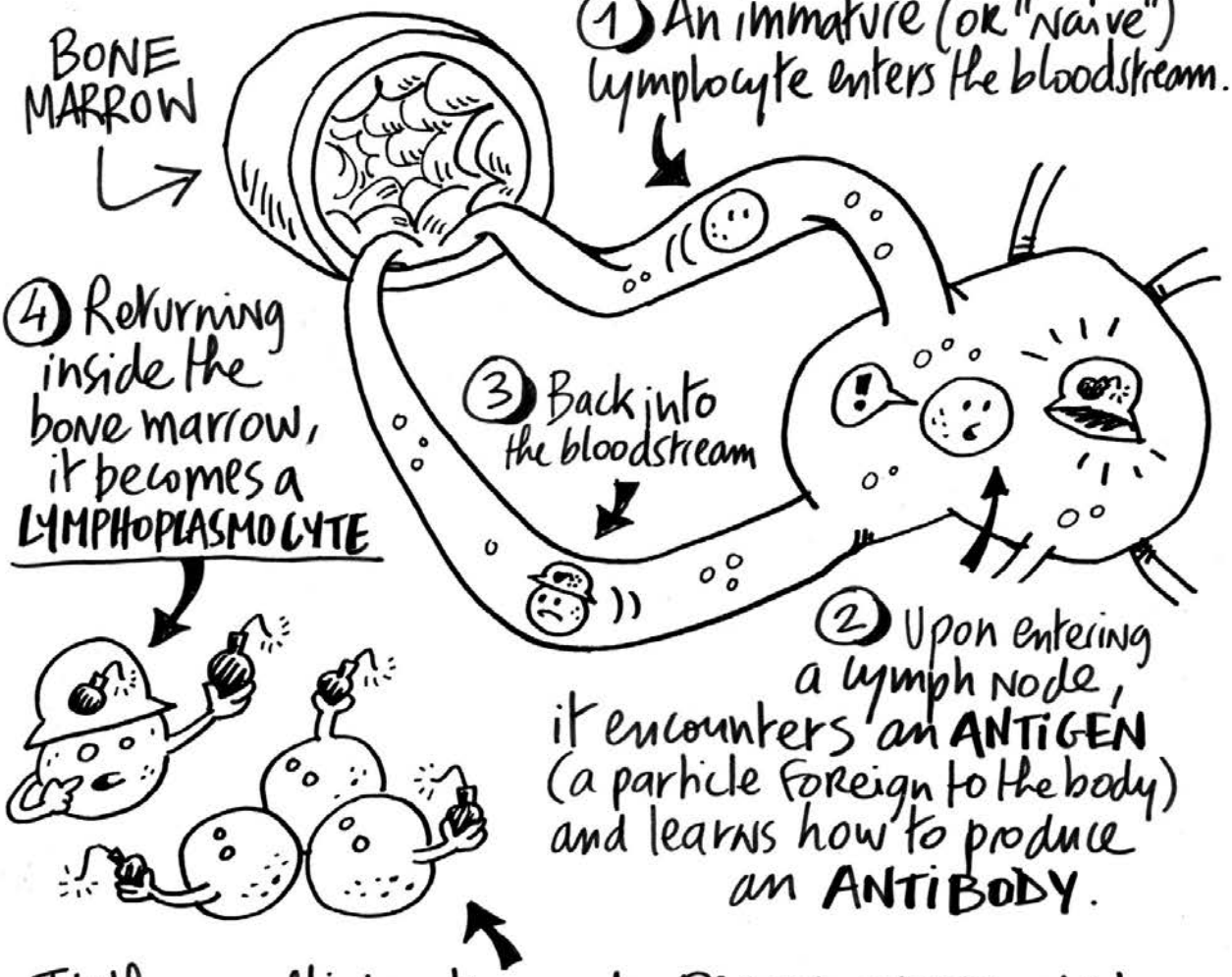


... PLASMOCYTES derived from LYMPHO-PLASMOCYTES that were LYMPHOCYTES coming from the BONE MARROW ...

... since, as you know, the various blood cells are "born" in the bone marrow. At first, a young lymphocyte is unexperienced, but it won't take long before it specialises ...

DIFFERENCIATION

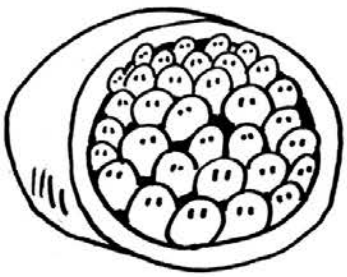
Here is how a LYMPHOCYTE becomes a LYMPHOPLASMOCYTE:



It then multiplies to create **PLASMOCYTES** which among other things will spread **IgM** type antibodies.

W.M.

Waldenström **M**acroglobulinemia is, inside the BONE MARROW, the uncontrolled increase of:



→ LYMPHOCYTES B,
→ LYMPHOPLASMOCYTES,
→ PLASMOCYTES derived from the same clone.

PLASMOCYTES will start producing an identical antibody (IgM) that will enter and crowd the bloodstream.

And that's what is called...

MONOCLONAL = multiplies identically	IMMUNOGLOBULIN = antibody

≠ POLYCLONAL

DIFFERENCES & SIMILARITIES

As in the case of CLL, lymphocytes are in excess, but there are numerous important differences:

The lymphocytes involved in **W.M.** are mainly (LYMPHO) PLASMOCYTES.

They remain inside the Bone Marrow and are thus scarce in the bloodstream.

They produce a single type of antibody (IgM) that proliferates in the bloodstream.

It's a disease that can remain asymptomatic: Like CLL, no treatment is needed if the patient remains symptom-free.

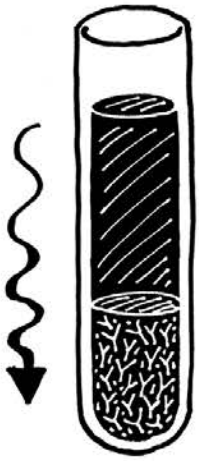
W.M. has a very slow development, 10 years can go by between the point of discovery and the moment treatment becomes necessary. Simple monitoring every 6 months with a blood test or a clinical exam is all that is needed.

THE TESTS

W.M. can be detected with three different chemical and biological tests.

#1: SEDIMENTATION RATE TEST.

A blood sample is taken to observe the rate at which a sediment falls: an abnormal quantity of antibody proteins tends to make the blood thicker.



#2: PROTEIN ELECTROPHORESIS

is done by a chromatographic machine that will differenciate and establish the blood sample's concentration in proteins, and evaluate a potential excess of IgM antibodies.

#3: BONE MARROW BIOPSY: Is done with the help of a special thin and hollow needle that will puncture either **THE STERNUM** or **THE PELVIC BONE** to take a bone marrow sample

The sampling lasts around one minute and is not more painful than a blood test, in most cases. The sample is then examined in a lab to evaluate its LYMPHOPLASMOCYTE concentration.

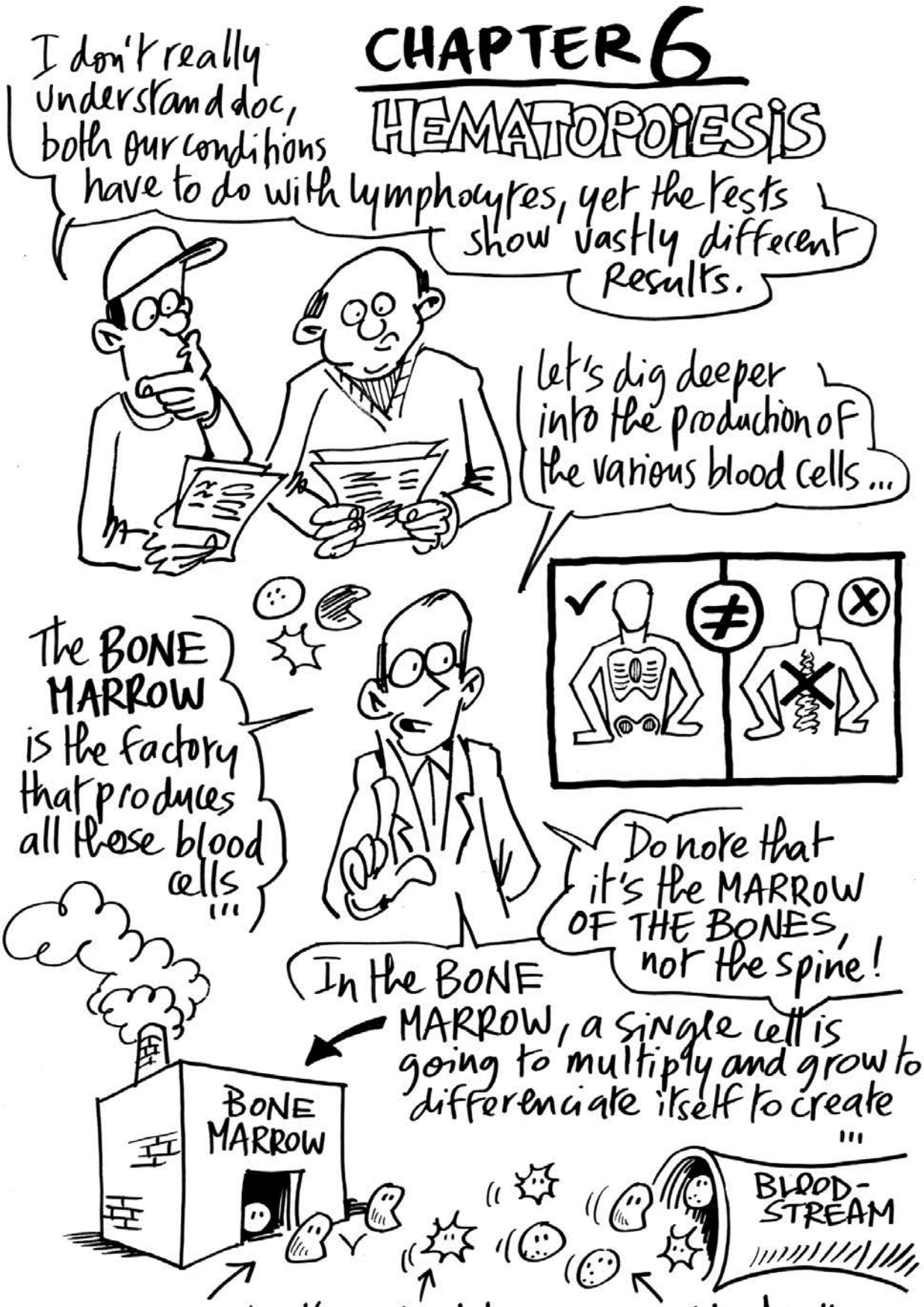
THE TREATMENTS

MONOCLONAL IMMUNOGLOBULIN increases the quantity of proteins in the bloodstream, increasing its viscosity and slowing its flow. This can in turn cause headaches and dizziness. Moreover, plasmocytes saturating the bone marrow will cut down other blood cells numbers, potentially causing anemia, exhaustion, shortness of breath, palpitations.

Chemotherapy or immunotherapy can be likely options, but there are other possibilities...

A large panel of new molecules and drugs can be combined to better suit the needs of each individual patient.

CHAPTER 6
HEMATOPOIESIS



There is a constant renewal of each of those cells to

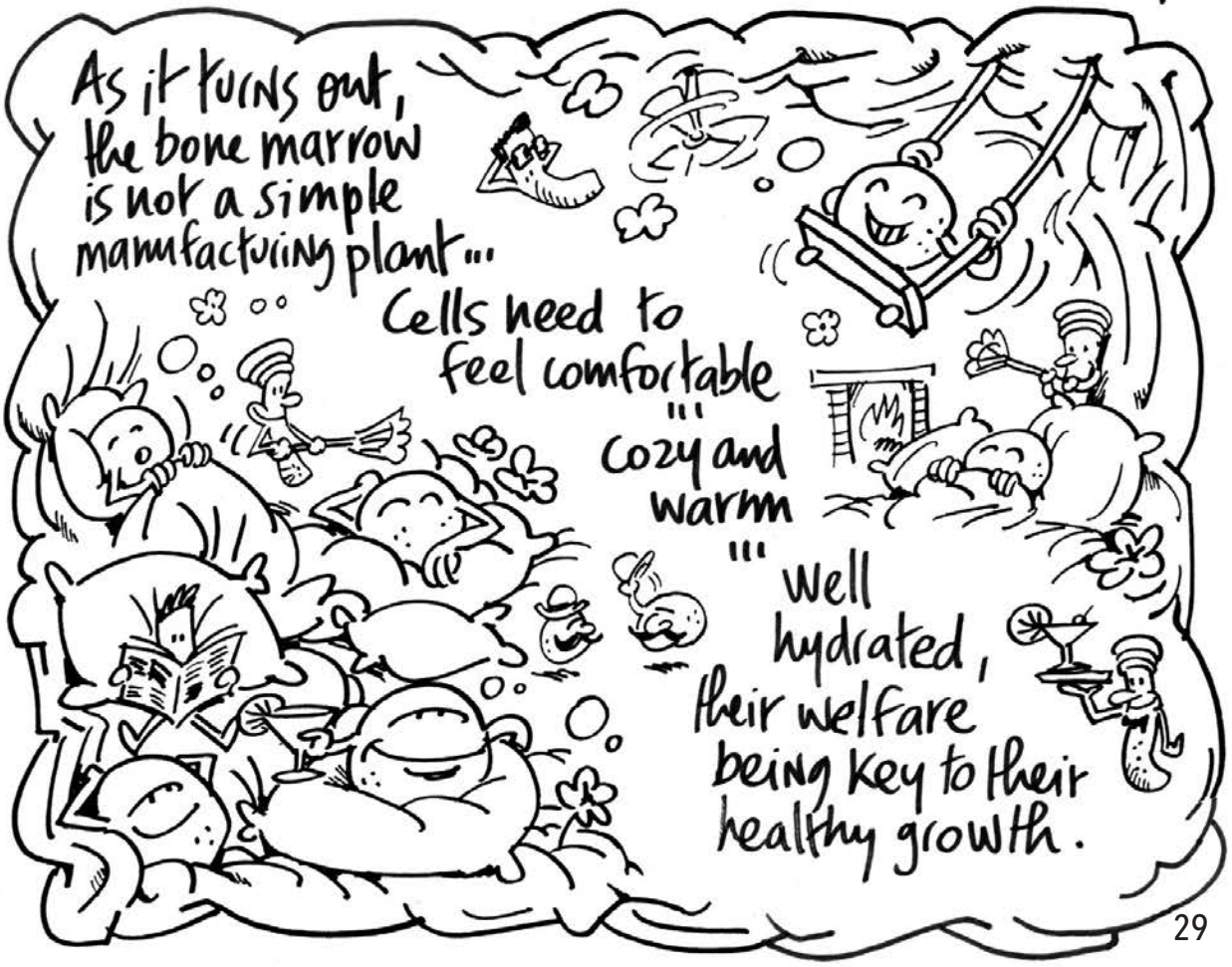
Replace cells with a limited lifespan.

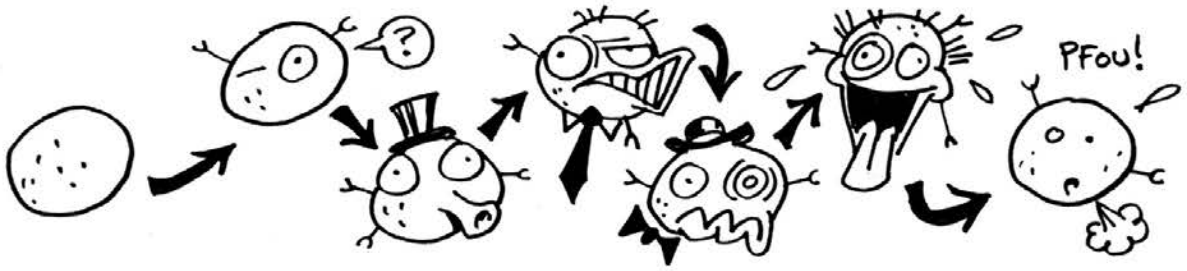
+/- 120 days for a red blood cell.

Adapt and compensate for deficiencies or diseases

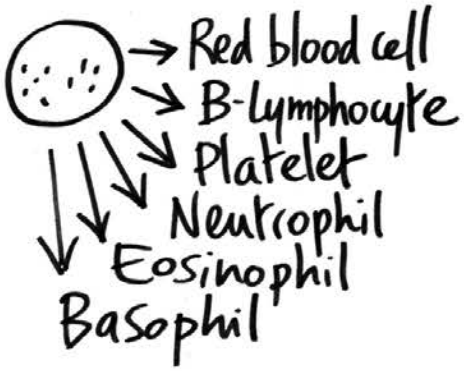
THE LOUNGE

Cells in the microenvironment need to be in a perfect setting for everything to function adequately.





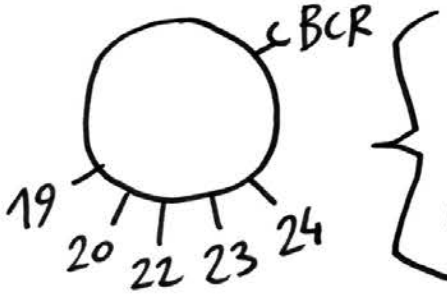
Amidst the other cells of the microenvironment and the communication proteins, each new cell will go through different states:



Once properly formed, the cell enters the bloodstream.

MARKERS

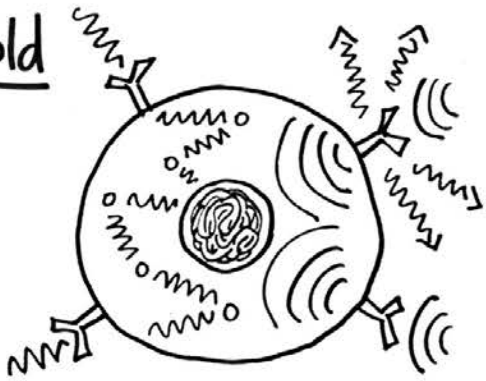
Along its journey, the cell will acquire or lose differenciation markers, small molecules on its surface



B lymphocyte not only has BCR but also many other different markers.

The markers' functions are manyfold

- Regulate each cell
- Transmit signals
- Activate the cell's inner signalisation channels.

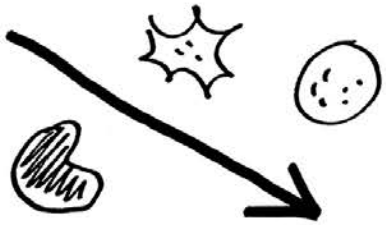


WITH CLL, a large amount of lymphocytes is found in the bloodstream and the bonemarrow.

They prevent the B lymphocytes (the ones producing antibodies) from properly functioning.

That's the reason why the antibody count is so low, and why there is a higher risk of infection. The electrophoresis shows this decrease.

If CLL lymphocytes are present in overwhelming numbers, it creates deficiencies in normal cells, causing anemia, neutropenia or thrombocytopenia.

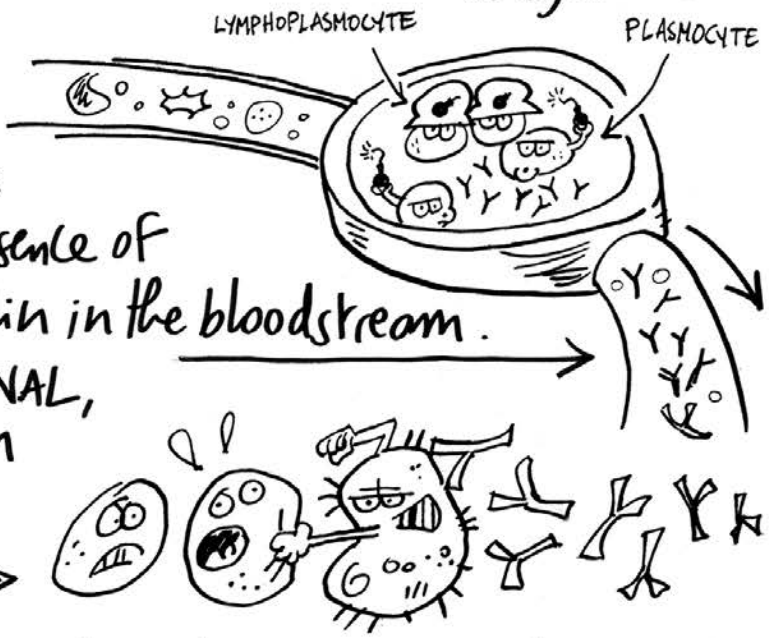


This is STAGE C and warrants the start of treatment.

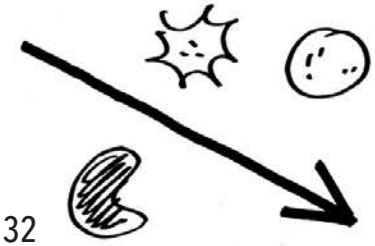
WITH W.M., the lymphocytes won't enter the bloodstream. They instead remain inside the BONE MARROW and become specialised cells, producing immunoglobulin M, or Igm.

Spikes visible on the electrophoresis will show the presence of this immunoglobulin in the bloodstream.

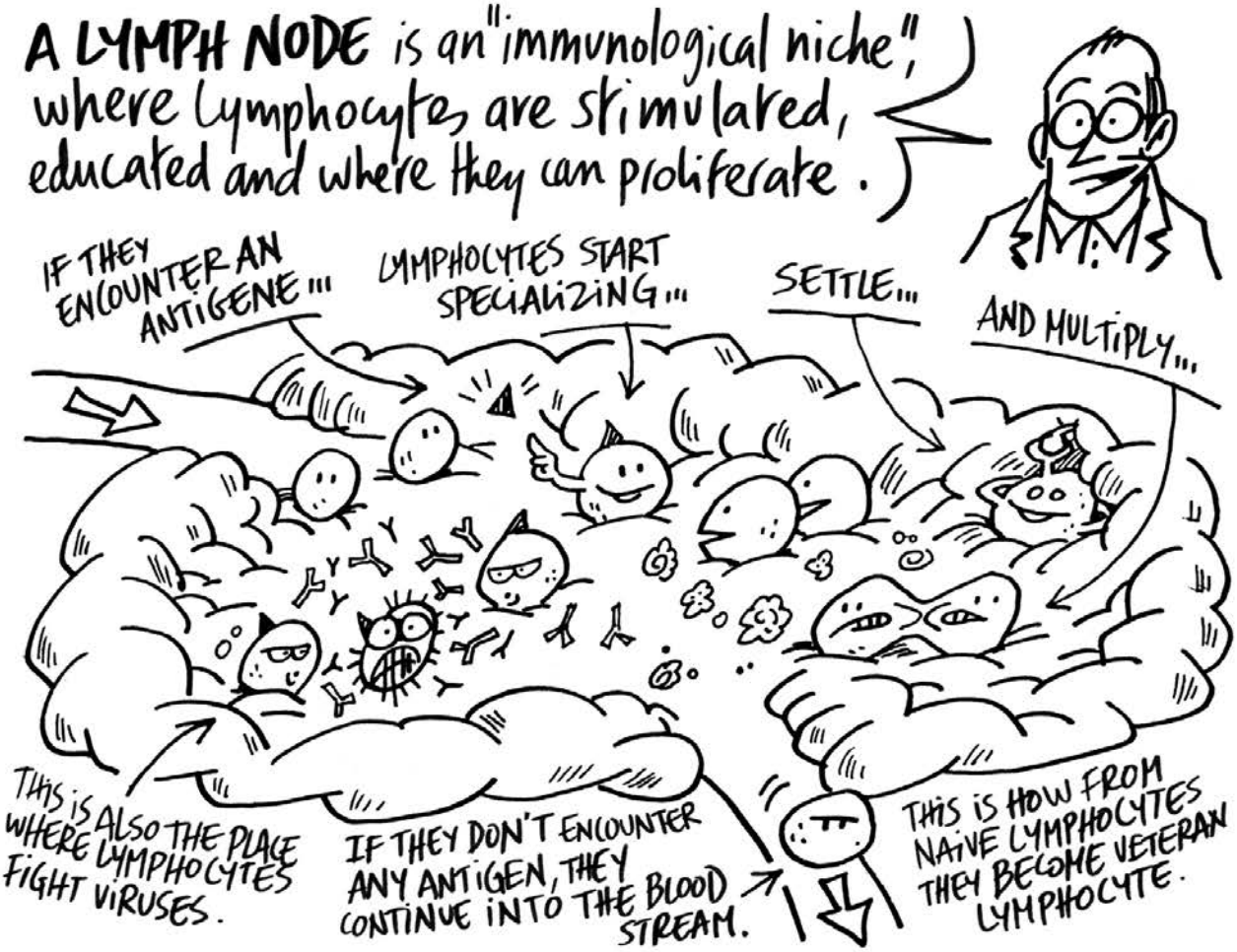
But being MONOCLONAL, this immunoglobulin is ineffective against infections!

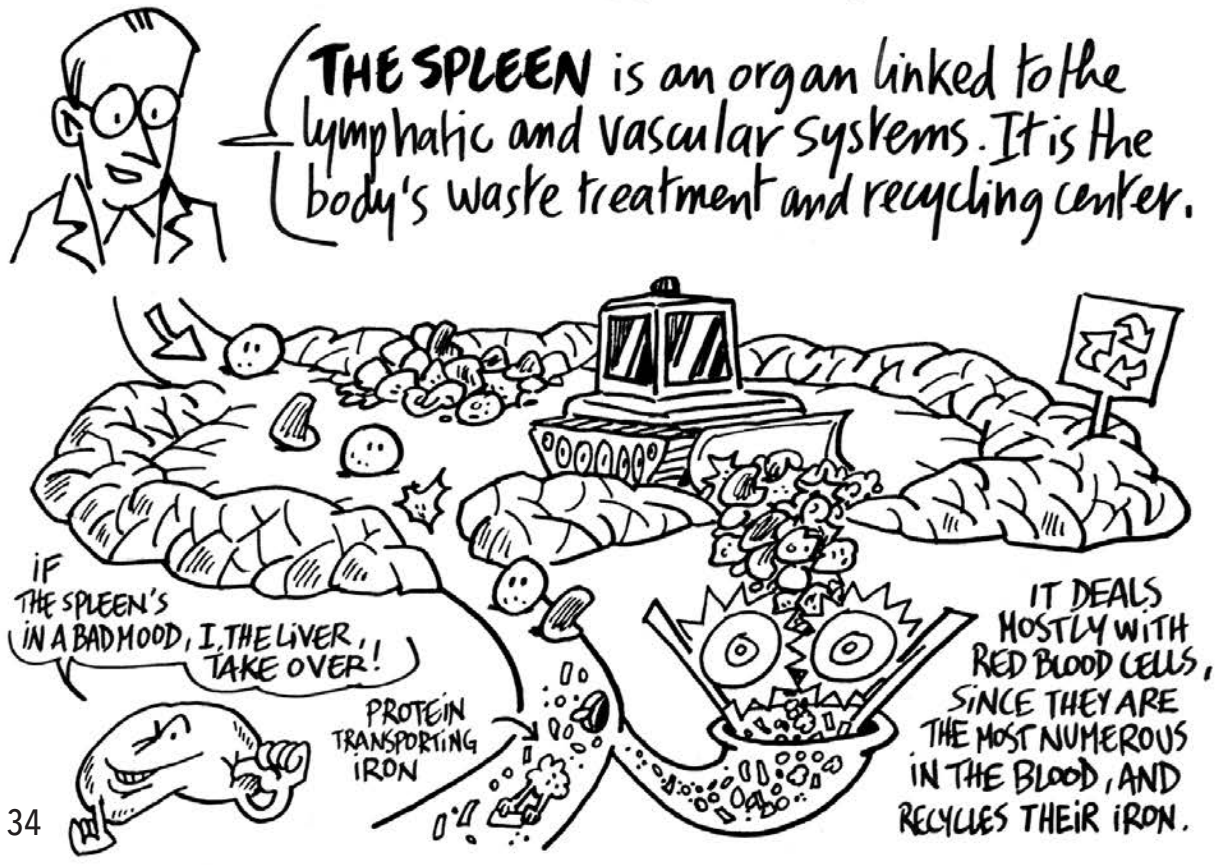


Furthermore, if those lymphocytes and plasmocytes are too numerous, they will prevent the bone marrow from producing normal blood cells, leading to the decrease shown by the CBC.



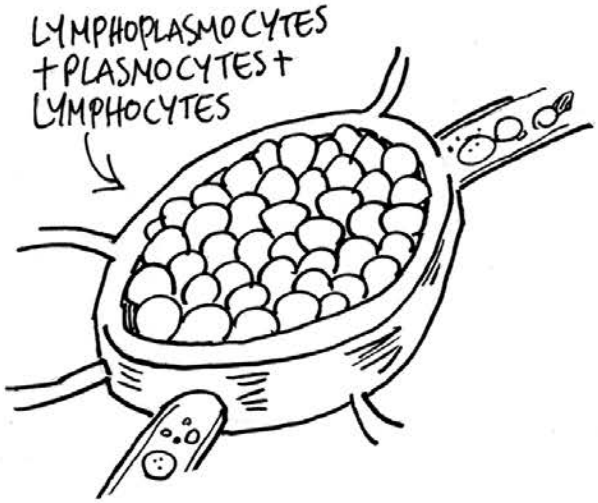

I don't get it then, why am I stuck with swollen lymph nodes?...
With CLL, the lymphocytes initialy produced in the BONE MARROW live as specialised cells in this hematopoietic niche...
BONE
FAT
BLOOD-STREAM
PROTEINS, NUTRIENTS, VITAMINS, IRON...
PLASMOCYTES & LYMPHO-PLASMOCYTES
NAIVE LYMPHOCYTES
LYMPH NODE
However, that's not where they like to multiply... CLL lymphocytes will move in the body throught the bloodstream straight to the lymph nodes.


A LYMPH NODE is an "immunological niche", where lymphocytes are stimulated, educated and where they can proliferate.
IF THEY ENCOUNTER AN ANTIGENE...
LYMPHOCYTES START SPECIALIZING...
SETTLE...
AND MULTIPLY...
THIS IS ALSO THE PLACE WHERE LYMPHOCYTES FIGHT VIRUSES.
IF THEY DON'T ENCOUNTER ANY ANTIGEN, THEY CONTINUE INTO THE BLOOD STREAM.
THIS IS HOW FROM NAIVE LYMPHOCYTES THEY BECOME VETERAN LYMPHOCYTE.


THE SPLEEN is an organ linked to the lymphatic and vascular systems. It is the body's waste treatment and recycling center.
IF THE SPLEEN'S IN A BAD MOOD, I, THE LIVER, TAKE OVER!
PROTEIN TRANSPORTING IRON
IT DEALS MOSTLY WITH RED BLOOD CELLS, SINCE THEY ARE THE MOST NUMEROUS IN THE BLOOD, AND RECYCLES THEIR IRON.

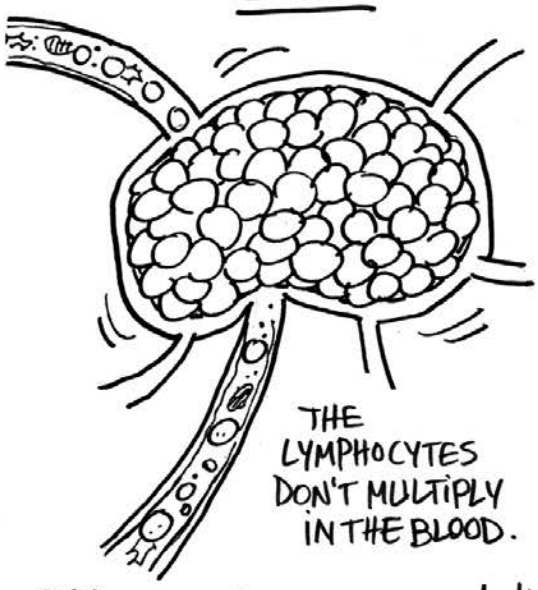
IN SUMMARY

WALDENSTROM MACROGLOBULINEMIA



Waldenstroom's macro-globulinemia causes an overproduction of plasmocytes that accumulate in the BONE MARROW, leading to decrease numbers of cells (anemia).

CLL



CLL causes an overproduction of lymphocytes that settle and build up inside the LYMPHNODE consequently inflating it.

It can sometimes inflate if there are too many CLL cells. In such a case, it gets bigger and eliminates platelets and red blood cells.

That's why CLL causes the lymph node to swell up and W.M. causes plasmocytes to multiply in the bone marrow, therefore hindering normal cell production.

CHAPTER 7

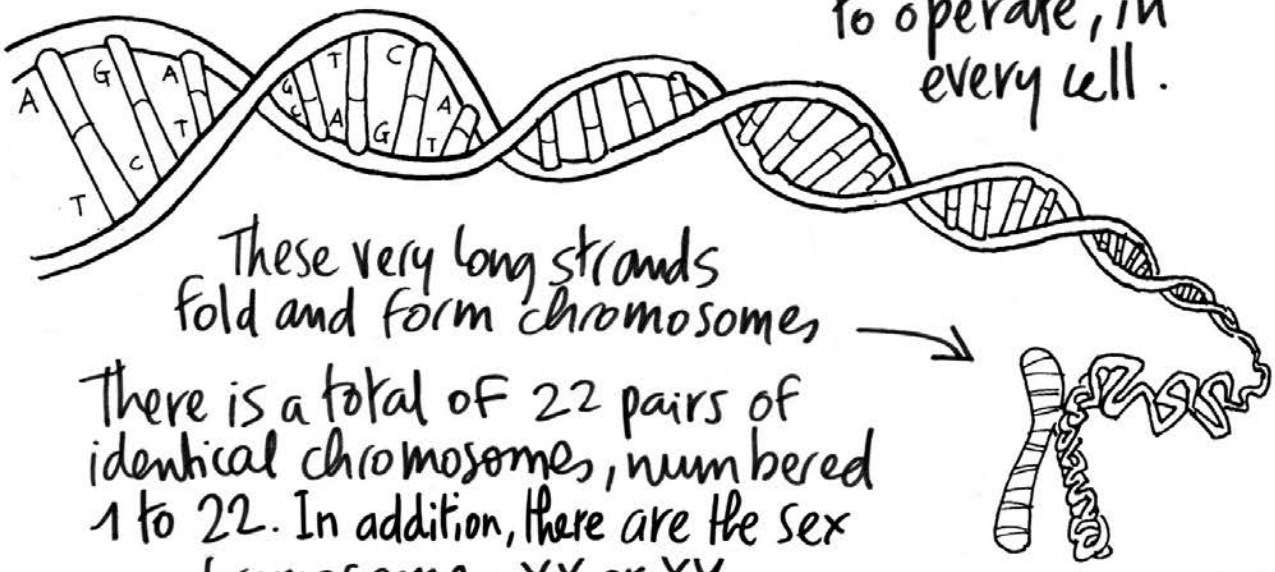
A BIT OF GENETICS

THE DNA

DNA is a molecule that is Found in all living cells.

It is made of two strands Facing each other, forming a double helix !!!

It contains all the informations required For the whole body to operate, in every cell.



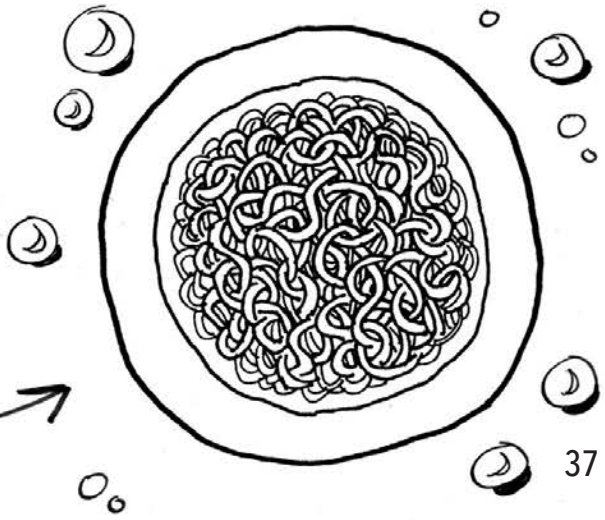
These very long strands fold and form chromosomes →

There is a total of 22 pairs of identical chromosomes, numbered 1 to 22. In addition, there are the sex chromosomes XX or XY.

THE MITOSIS

Our cells are constantly renewed by self-replicating

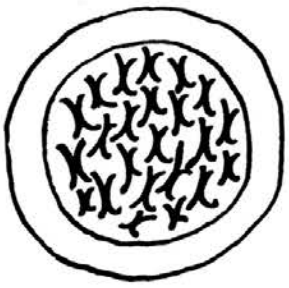
The chromosome are found inside the NUCLEUS of cells, as uncondensed, very long threads of DNA resembling balls of yarn. →



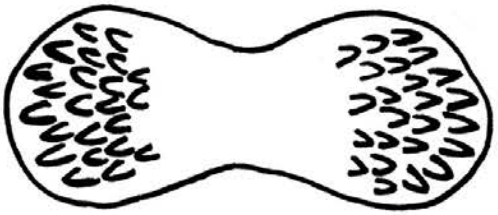
The mother cell needs to replicate its DNA in order to divide itself in two daughter cells, identical to each other !!!

This is mitosis

1. The chromosomes will condense and get replicated, then align for metaphase.



2. They split and migrate to the cell's poles.



3. Dividing itself in the middle, the mother cell becomes two identical daughter cells.

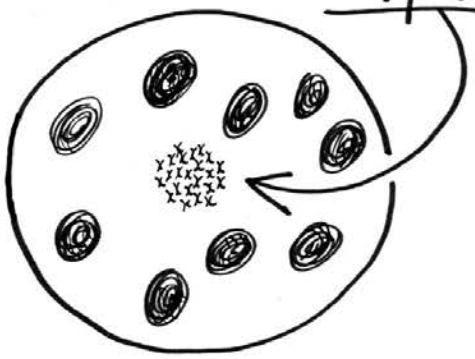
It's during metaphase that the condensed chromosomes are studied by cytogenetic analysis.

Your blood sample is mixed in a nutrition liquid for three days.

SOME CELLS DIVIDE THEMSELVES

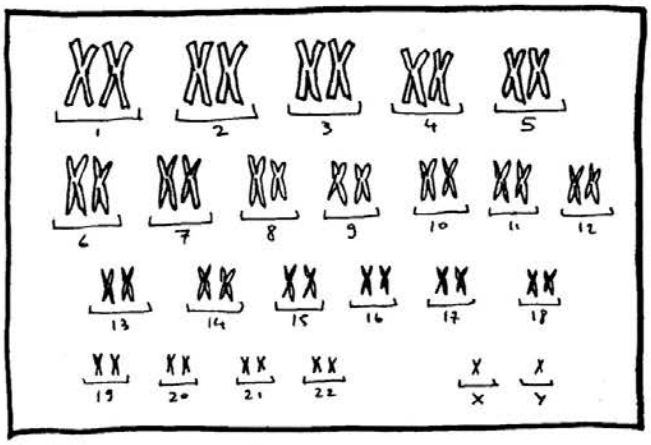
37°C / 99°F

The cell divisions are abruptly stopped and they are broken up. Shocked in metaphase, the cell chromosomes are condensed and well prepared for visual analysis.



THE CARYOTYPE

After a photo is taken, a software sorts the chromosome pairs in a table, THE KARYOTYPE, allowing me to analyse and compare them.

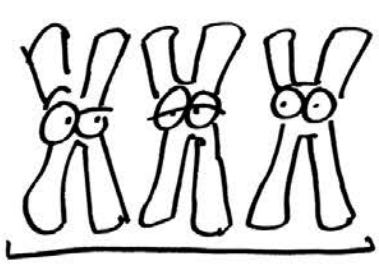


There are 22 pairs of identical chromosomes, numbered from 1 (the largest) to 22 (the smallest) + The sex chromosomes pair, XX for females and XY for males.

THE ANOMALIES

Sometimes, during mitosis, some cells don't duplicate identically ... some have a "SURVIVAL ADVANTAGE", meaning that they are stronger and that their proliferation goes unregulated by the body.

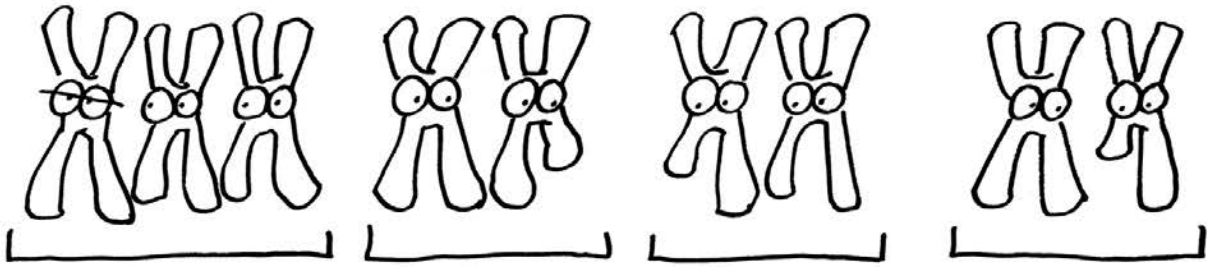
KARYOTYPE ANALYSIS ALLOWS THE TARGETING OF **GENETIC DISORDERS**



TRISOMY
3 chromosomes instead of 2

DELETION
A portion of the chromosome is missing

In the case of **CLL**
4 TYPES OF ANOMALIES are found:



TRISOMY 12 | 13Q DELETION | 11Q DELETION | 17P DELETION

The latter is special because the cells bearing this anomaly are **UNAFFECTED BY FLUDARABINE**

THESE ANOMALIES ARE ACQUIRED...

They are only found in the CLL LYMPHOCYTES.

≠

...NOT INNATE.

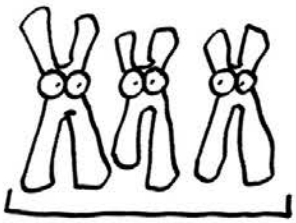
They are not found in every cell of the body since birth, as they would in case of the DOWN SYNDROME.

THERE ARE OTHER ANOMALIES...

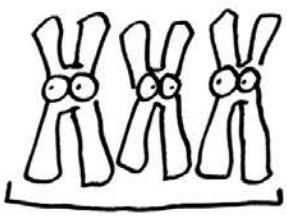
TRANSLOCATION is the abnormal repair of a broken chromosome with a fragment from another chromosome. THEY ARE RARELY FOUND IN CLL.

WITH W.M. we can also find chromosomic anomalies in the bone marrow's lymphoplasmocytes, most notably!

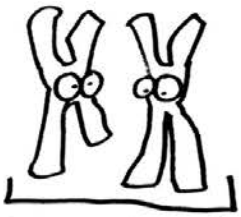
DELETION 6q



TRISOMY 4



TRISOMY 18



DELETION 17p (same with CLL)

These anomalies are of no consequences on the evolution or treatment of W.M.

GENETIC ANOMALIES

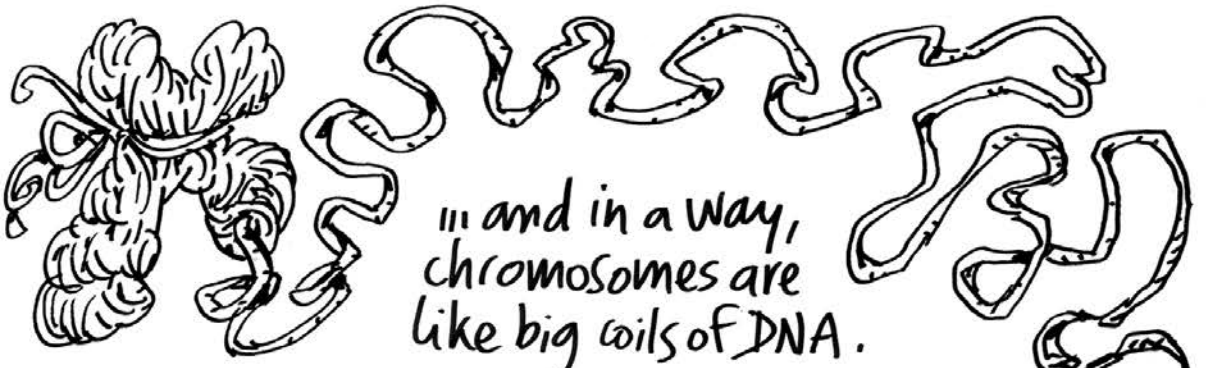
Aside from chromosome analysis, I wanted to make sure there wasn't an even smaller anomaly, A MUTATION, which would prevent the p53 protein to function normally. This isn't noticeable with karyotype analysis: it's GENETICS!

So what's a genetic anomaly?

It's an anomaly located inside a gene, namely on the DNA strand.

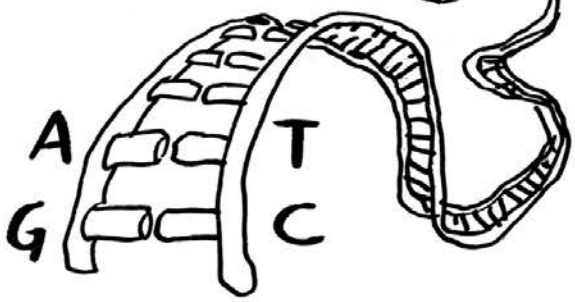
DNA

We've seen that all the information is stored in the DNA...



...and in a way, chromosomes are like big coils of DNA.

DNA, when uncoiled, is actually composed of two facing strands, each made of NUCLEOTIDE sequence.



There are 4 NUCLEOTIDES corresponding 2 by 2 :

A = Adenine
T = Thymine
G = Guanine
C = Cytosine

IT'S A 4 LETTER LANGUAGE

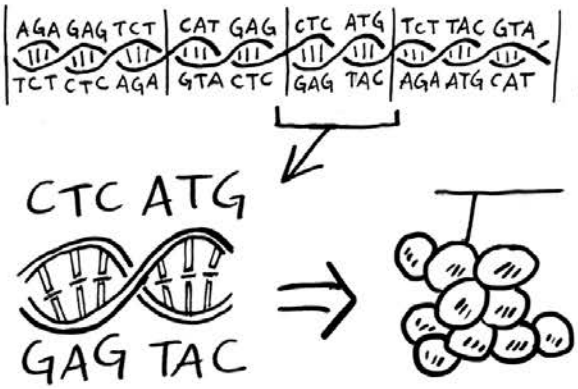
A G
| |
T C

The 2nd strand of the double helix is a mirrored copy of the 1st strand

A G C C T A A T
| | | | | | | |
T C G G A T T A

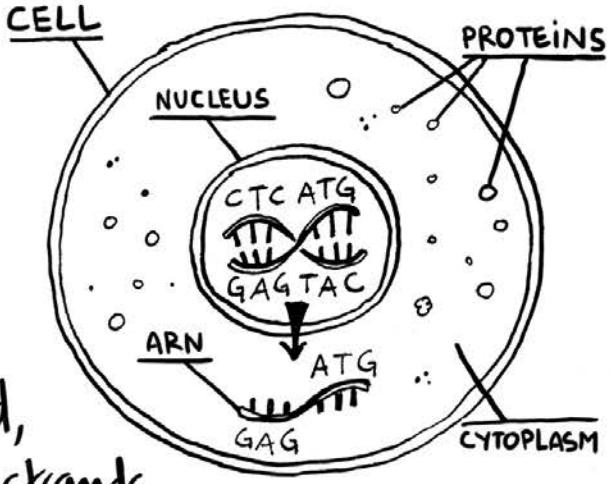
GENES & GENETIC CODE

The DNA molecules are subdivided into GENES. There are 25,000 different ones.

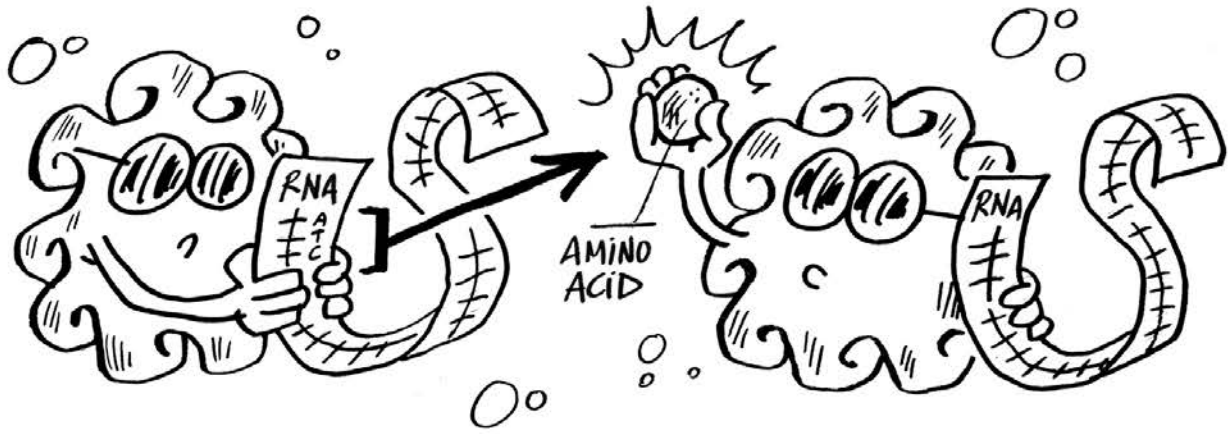


Each gene is a "recipe" allowing for the production of ONE PROTEIN with a specific function in the cell.

To produce this protein, the gene will be copied as RNA



Acting as the messenger, the RNA only has one strand, identical to one of the DNA strands.



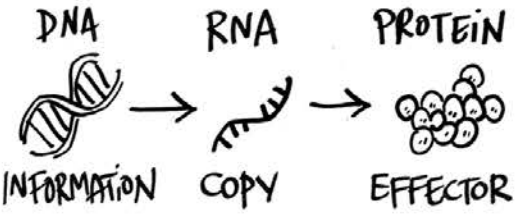
Once out of the nucleus, the RNA is handled by complex proteins, the RIBOSOMES, which will translate it into proteins. RNA is read in strings of 3 letters called CODONS. For each CODON, the RIBOSOME will add one amino acid. Amino acids are the building blocks of proteins.

THAT'S THE GENETIC CODE

A CODON STOP marks the end of the DNA.

Once translated, the amino acids form a protein matching the gene recipe.

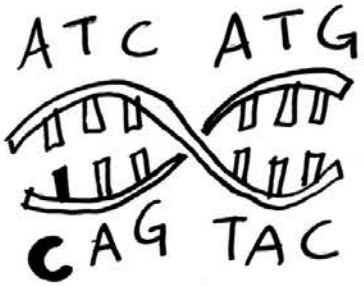
MUTATIONS



A mutation is a nucleotide anomaly (A,T,G,C) causing an abnormal expression of the gene, and thus of the protein.

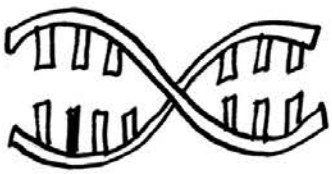
A one letter error in the code can offset the whole chain. There are 3 different anomalies:

POINT MUTATION



One nucleotide is changed and the CODON's meaning changes.

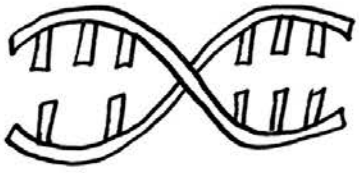
INSERTION



Extra nucleotide

This will shift the whole chain by a notch when getting copied. It's called a frameshift.

DELETION



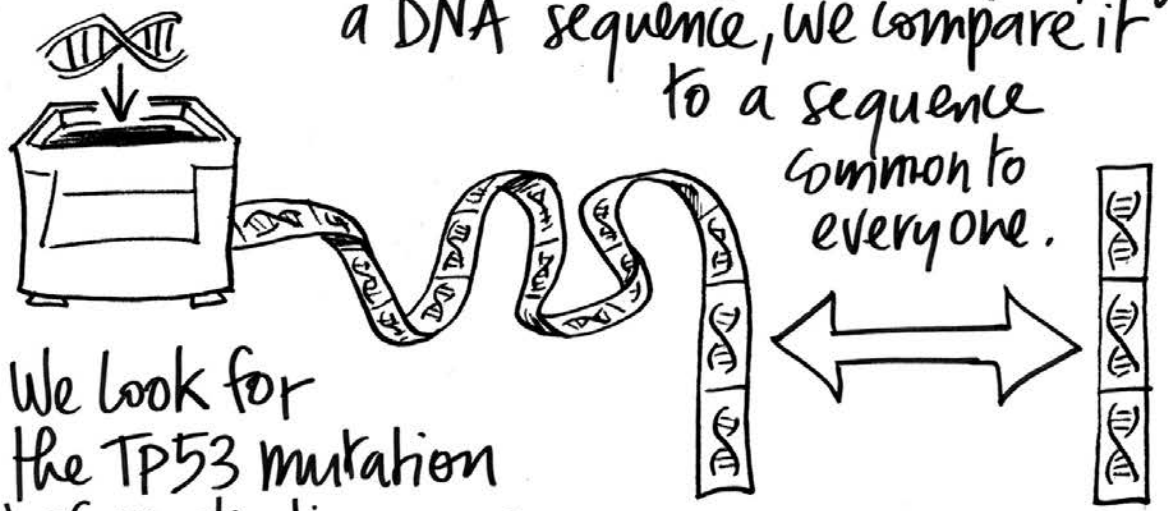
Missing Nucleotide

As they are read 3 by 3, the codon as well as the following amino acids will be different.

That's how incomplete or abnormal proteins can appear.

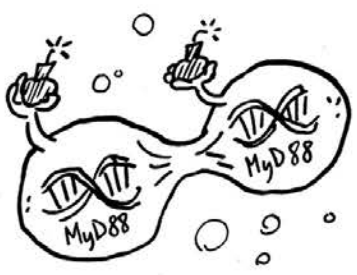
With CLL, we are especially looking to the **TP53 MUTATION** which has effects similar to the **17p DELETION**.

We use MOLECULAR BIOLOGY techniques to spot mutations: after extracting and amplifying a DNA sequence, we compare it to a sequence common to everyone.



We look for the TP53 mutation before starting any treatment, as cells carrying this anomaly are UNAFFECTED by FLUDARABINE.

WITH W.M., following the exact same principle, we'll look for a mutation on the MyD88 gene. This mutation helps diagnose the disease and has no UNFAVORABLE significance. It's found in the majority of patients and contributes to the wild proliferation of plasmocytes.



CHAPTER 8

PRE-TREATMENT ASSESSMENT

BEFORE STARTING CHEMOTHERAPY, A FULL ASSESSMENT IS NECESSARY.

We'll start with a FULL BODY SCAN to have a look at all your lymph nodes.

You see, they're also present in the thorax and abdomen, and we need to check if they've swollen before treating.

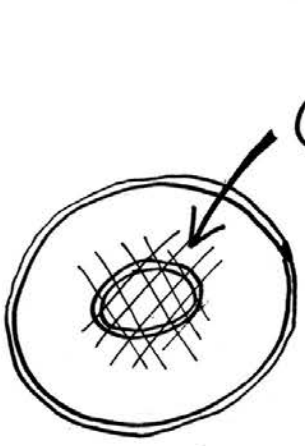
Then we'll run a BLOOD TEST to check the health of your kidneys, liver, etc... since chemo will have them working overtime.

Finally we'll check for any infection, and especially for viruses like hepatitis, as your immune system will be impaired by chemo for some time.

CHAPTER 9
TREATMENTS

IMMUNOCHEMOTHERAPY

Your tests results show a deletion of the 13q chromosome, with no adverse effect. There is no Tp53 anomaly so you can get chemotherapy paired with an antibody that targets your CLL cells.



The **FCR** (Fludarabine, Cyclophosphamide & Rituximab) and **BR** (Bendamustine & Rituximab) treatments create fractures in the DNA of the leukemic cell nucleus, preventing them from duplicating, producing proteins and generally functioning normally.

Regarding SIDE EFFECTS, chemo isn't very selective on the cells it targets, also targeting normal lymphocytes protecting the body. So chemo will make you more vulnerable to infections for some time, making some medication mandatory.

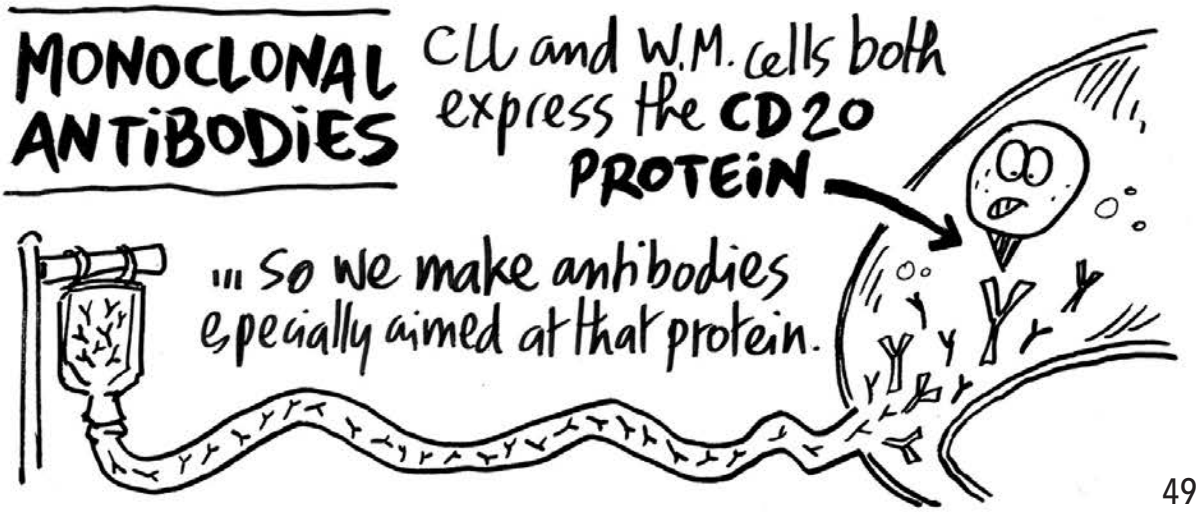
Chemo can induce nausea, but we now have very effective drugs to prevent that.

I also want to point out that those chemotherapies won't provoque any hair loss.

Chemo and antibody-immunotherapy are usually combined for higher efficiency.

MONOCLONAL ANTIBODIES

CLL and W.M. cells both express the **CD20 PROTEIN**



During the intravenous infusion, the antibodies will bind the CLL lymphocyte, and make the chemo action more efficient on the cells.

During the treatment you'll receive a few injections to keep your other white blood cells (the polyclonal ones) from dropping too low ...

Despite all this, you might still catch a FEVER. You must immediately see your doctor who'll prescribe antibiotics if necessary ...

While on chemo, don't let a fever linger.

If promptly treated, the infection will be quickly stopped.

6 FCR TREATMENTS LATER: STAY VIGILANT.

MINIMAL RESIDUAL DISEASE

IT'S BEEN 3 MONTHS SINCE THE END OF VINCENT'S LAST TREATMENT...

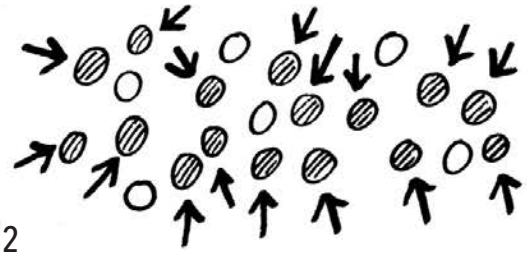
Doc, as part of a protocol, I need to undergo Residual disease testing... What is it exactly?

MRD TESTING.

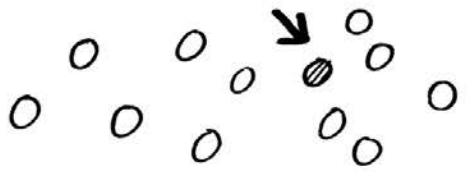
Minimal Residual Disease, is a treatment follow up to establish precisely if there are any remaining traces of the disease in the bloodstream or the bone marrow.

Kind of like looking for a needle in a haystack.

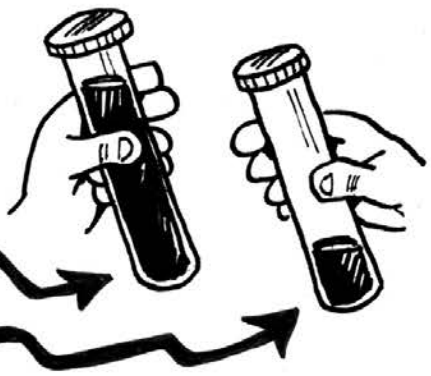
BEFORE treatment, we analyse the features of unhealthy cells...



...which will allow to distinguish them from healthy cells AFTER treatment.



AFTER treatment, TEST SENSITIVITY depends on the amount of blood sampled and tested, since there are higher chances of finding something **HERE** than **THERE**



TECHNIQUE

MRD testing is done using two different techniques:

#1 FLOW CYTOMETRY

(also used to diagnose CLL, as we've seen before.)

It's the surface antigens on lymphocyte that we're interested in. In the case of CLL, some markers are IN EXCESS or MISSING.

We add FLUOROPHORE stained antibodies to the white blood cells.

I'm doing it just right, no mess...

Each lymphocyte emits a very specific fluorescence for the FLOW CYTOMETER to identify. This way, we can find 1 cell in the middle of 100.000 others. With M.M, we follow treatment efficiency on the IgM spike of electrophoresis.

UTILITY & SUBTLETIES

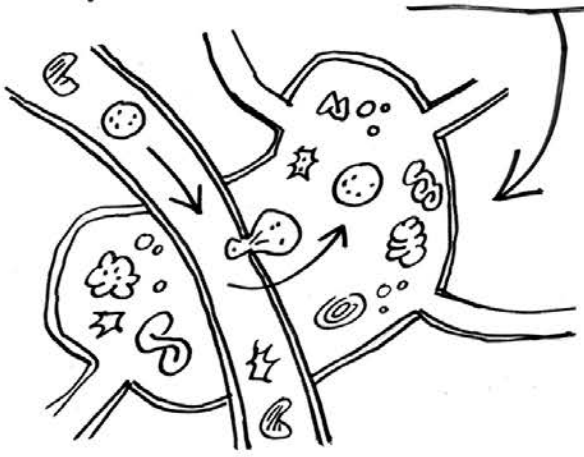
SIGNALING INHIBITORS

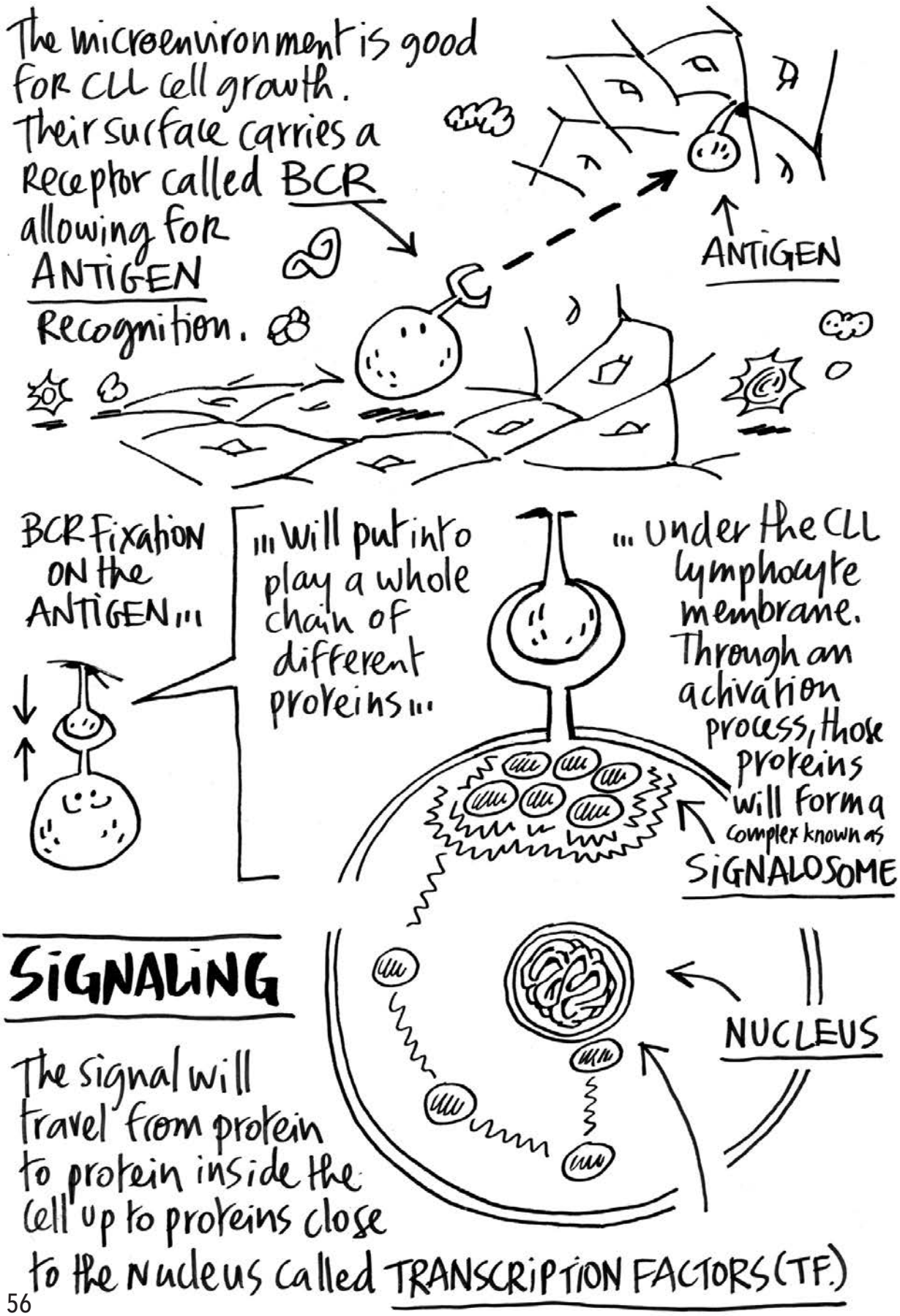
IN THE WAITING ROOM, VINCENT MEETS JACKIE

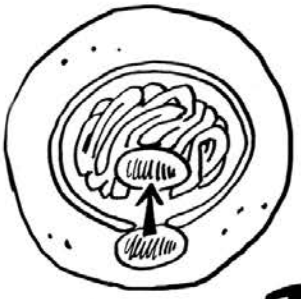
ANTIGENS & B-CELL RECEPTORS (BCR)

Traveling in the bloodstream, the lymphocytes are guided by the veins to the LYMPHNODES

Different types of cells inhabit the lymph nodes and form a tissu called The MICROENVIRONMENT.




The microenvironment is good for CLL cell growth. Their surface carries a receptor called BCR allowing for ANTIGEN Recognition.
ANTIGEN
BCR Fixation ON the ANTIGEN...
... will put into play a whole chain of different proteins...
... under the CLL lymphocyte membrane. Through an activation process, those proteins will form a complex known as SIGNALOSOME
SIGNALING
NUCLEUS
The signal will travel from protein to protein inside the cell up to proteins close to the nucleus called TRANSCRIPTION FACTORS (TF.)



The TFs will enter the nucleus through the membrane, bind to its DNA and give orders.

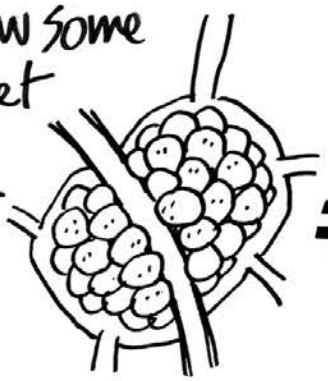
THUS, THE CLL CELLS WILL:

First settle and stay in the lymph node.

Then multiply and proliferate

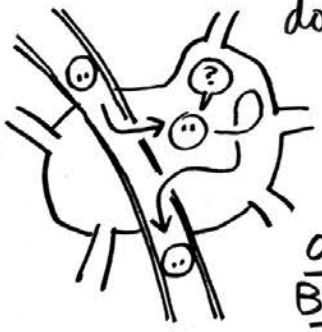
... in the lymph node.

That's how some people get SWOLLEN LYMPH NODES



≠

For patients whose CLL doesn't progress,



THE CELLS DON'T SETTLE AND MOVE ON INTO THE BLOODSTREAM.

SIGNALING INHIBITORS

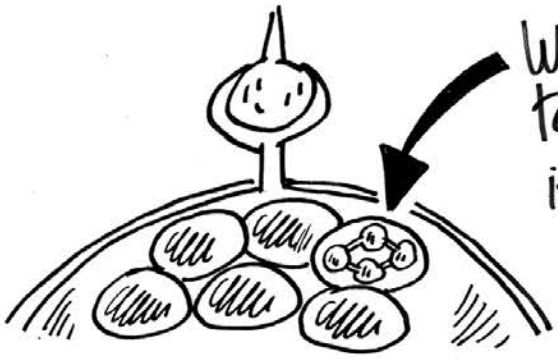
The CLL cells are getting targeted by new drugs that will impact one of the SIGNALOSOME PROTEINS.

The BTK protein will be targeted by the inhibitor

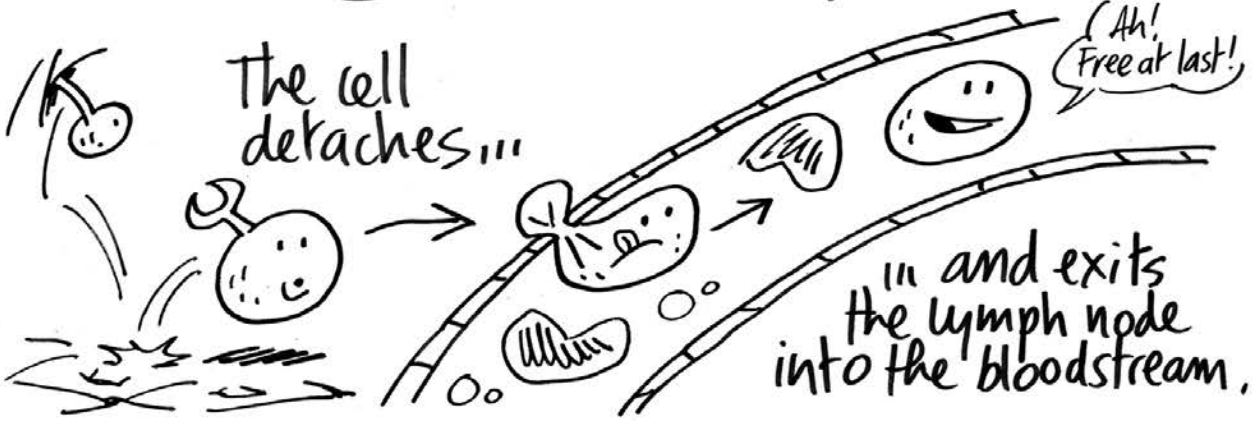
IMBRUVICA®
(Ibrutinib)

The PI3K protein will be targeted by the inhibitor

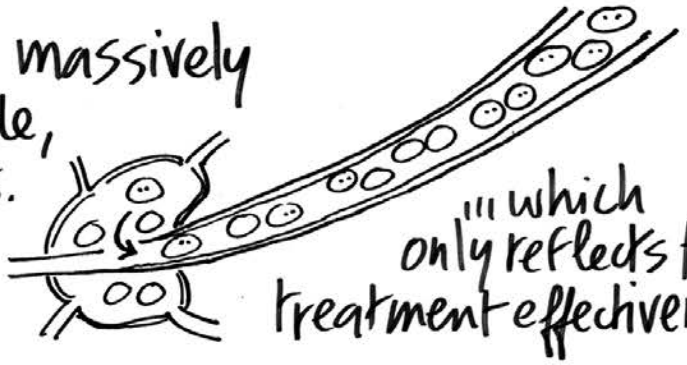
ZYDELIG®
(Idelalisib)



With an inhibitor binding to a protein, signal transmission is blocked. The cell stops producing the components necessary to its proliferation.



This way, CLL cells massively leave the lymph node, which rapidly deflates. They are now very numerous in the blood...



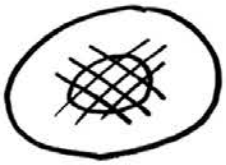
... which only reflects the treatment effectiveness!

ADVANTAGES

This way CLL cells will progressively die and be eliminated...

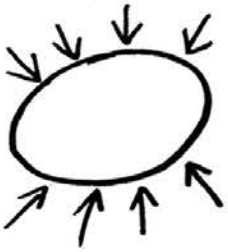
These new treatments are very effective, even on patients with cell anomalies that resist normal chemotherapies, like chromosome 17 anomalies.

NEW TREATMENTS



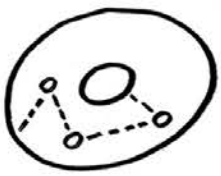
#1 CHEMOTHERAPY

targets the cellular division mechanism by breaking down the DNA in the nucleus.



#2 IMMUNOTHERAPY & MONOCLONAL ANTIBODIES

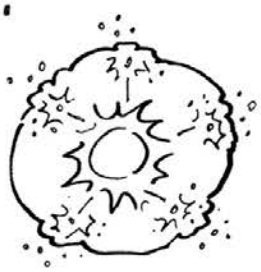
operate directly on the cell surface to destroy them.



#3 SIGNALING INHIBITORS

block signals transmitted inside the cytoplasm, from protein to protein, up to the nucleus.

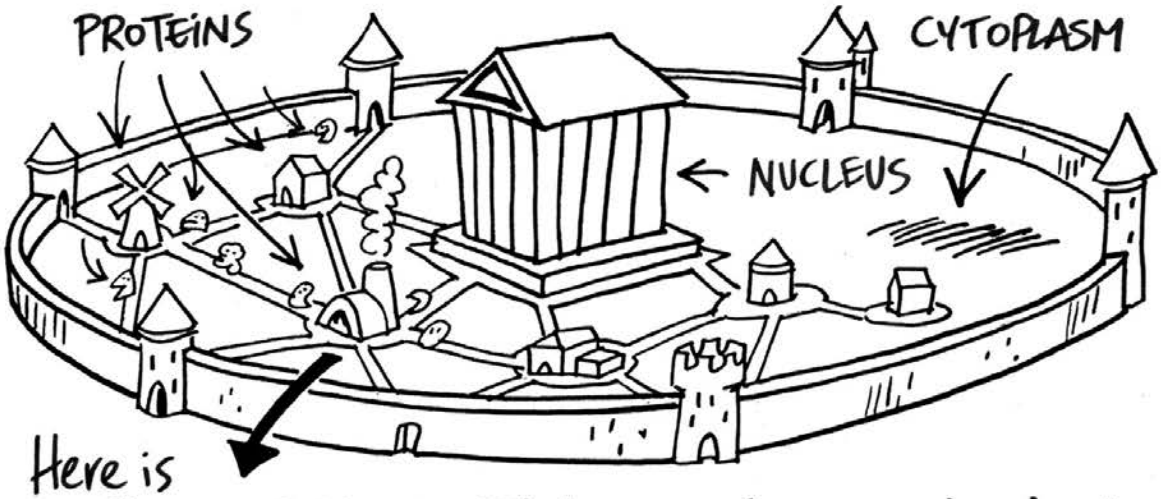
Furthermore, NEW DRUGS called **CLAX** target another cellular process: APOPTOSIS.



In other words, organised auto-destruction, a natural and necessary physiological process.

THE FORTRESS

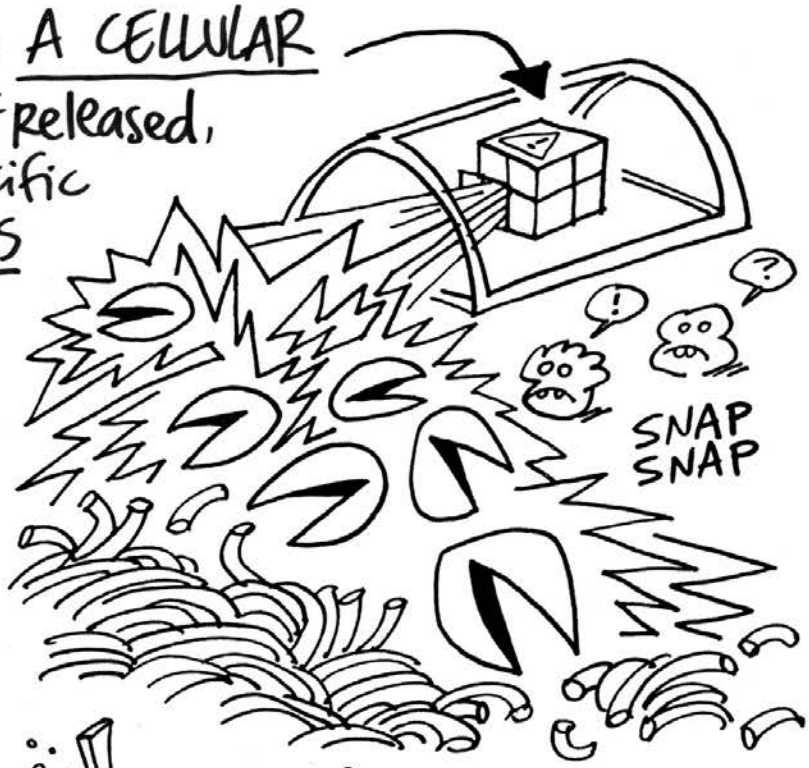
In order to understand the way these drugs work, let's say a cell is like a fortified city.



Here is A MITOCHONDRION: It transmits signals, just like the rest of the main hubs of the cell (cellular organs or organels). But also ...

(1) It plays a part in digesting the nutriments and turning them into ATP (Adenosine Triphosphate produced by Phosphorylation), in other words ENERGY →

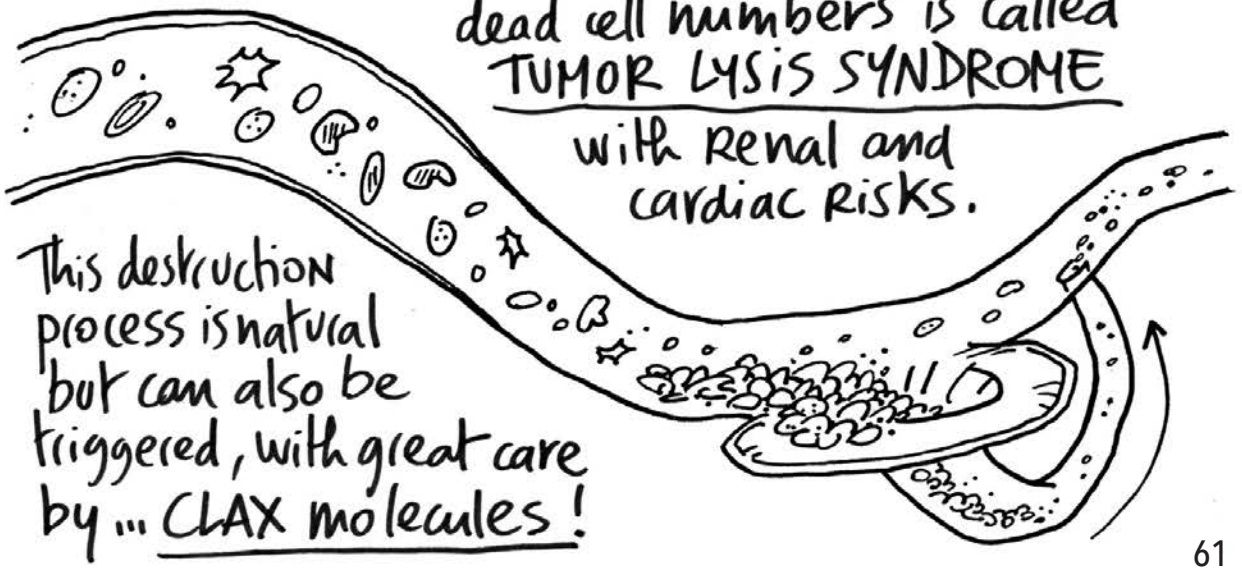
(2) It contains A CELLULAR POISON, which, if released, will activate specific proteins, CASPASES that will cut up the cell's DNA.



This CYTOTOXIC destruction is also known as APOPTOSIS.

Once the cell is destroyed, the kidneys filter everything and recycle the left-overs in the blood stream.

A case of overwhelming dead cell numbers is called TUMOR LYSIS SYNDROME with renal and cardiac risks.



This destruction process is natural but can also be triggered, with great care by ... CLAX molecules!

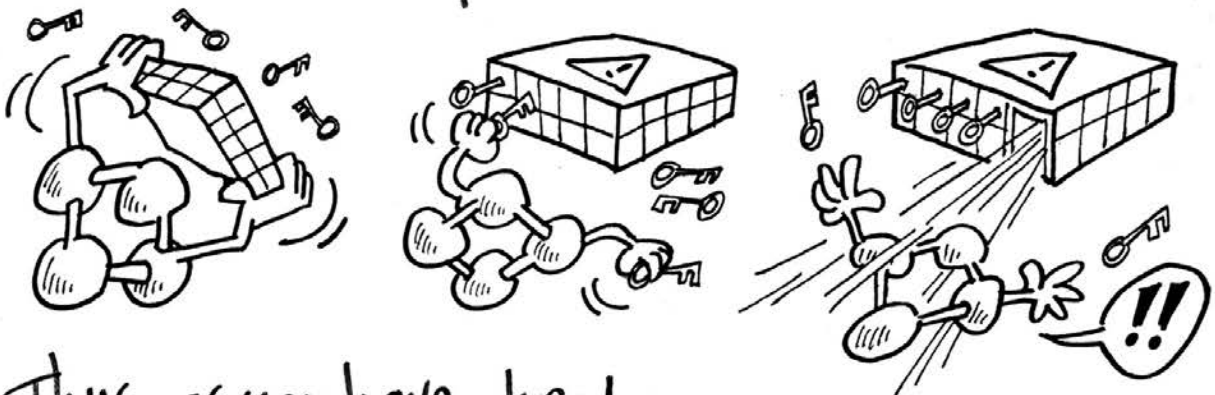
THE DOORS

The aforementioned poison is located in a membrane surrounded by BCL-2. They are the Doors.

SOME OPEN	OTHER STAY CLOSED
BAX, BAK ⚠	Bcl-xL A1 Bcl-w Bcl-2 etc...

In a healthy cell, there aren't many BCL-2 and the cell can self-destruct when its time has come. But with CLL, cancerous cells have a lot of them, and they are all stuck.

CLAX have the ability to dislodge the keys... ... that will help opening the BAX or BAK doors.



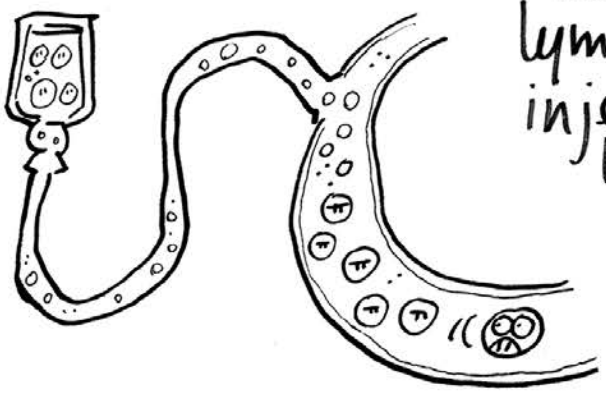
Thus, as you have already guessed, leading to the cancerous' cell destruction.

CLAX molecules are therapeutic novelties with numerous advantages: they're not only very effective, they can also be paired with other treatment protocols or when IBRUTINIB is ineffective or not recommended.

(Wow! Fascinating!) But wait, there's more!

There's a brand new procedure that will be available to patients whose CLL has progressed in spite of the treatments!

CAR-T CELL THERAPY



Based on your body's lymphocytes, we will manufacture killer lymphocytes that, once injected, will destroy leukemic cells ...

ALL THESE NEW TREATMENTS ARE VERY ENCOURAGING!

NOW & TOMORROW...

VINCENT, JACKIE, HARVEY AND GEORGE REGULARLY CHAT ON MESSAGE BOARDS...

Vincent's treatment ended 18 months ago; he's healing and doesn't take any more drugs...

Harvey doesn't have any health issues, his lymphocyte count rises slowly but with no swollen lymph nodes, he doesn't need treatment...

Jackie makes sure to take her ZYDELIG (Idelalisib) pills everyday. She found her rythm and is very happy this way.

George is also doing very well. He's taking his mind off things on a sunny beach now that his treatment is over.

We're in complete remission, without being cured for good, but it's so encouraging to see all these new treatments coming up!...

I spoke to someone who received FCR therapy 10 years ago and he's still in great shape...

I saw on the internet American patients undergoing IBRUTINIB therapy for more than 5 years who are perfectly healthy!


Thanks to the hard work of all health care professionals and the collaboration of all stakeholders: patients, users, doctors, researchers... progress is made everyday, and you, dear reader, will benefit from it!
So... to be continued!
NT 2018

QUIZ

1) Does everyone have lymph nodes even without having a disease?

a. Yes
b. No

2) Regarding leukocytes:

a. They are all the white blood cells
b. Granulocytes are leukocytes
c. Lymphocytes are leucocytes
d. Platelets are leukocytes
e. Monocytes are leukocytes

3) The lymphocytes circulate:

a. In the blood
b. In the lymph nodes
c. In the spleen
d. In the bone marrow

4) Gümprecht shadows are mangled red blood cells.

a. Yes
b. No

5) The necessary tests to diagnose CLL are:

a. CBC (or FBC)
b. Peripheral blood Immunophenotyping
c. Bone Marrow Biopsy

6) CLL is classified in 3 stages. Stage C is the swelling of lymph nodes in several areas of the body.

a. Yes
b. No

7) With CLL, you must get a flu shot.

a. Yes
b. No
c. It depends on the cases

8) Protein electrophoresis allows to see:

a. A decreased gamma globulin level in CLL
b. A peak in Waldenström's Macroglobulinemia

9) IgM in Waldenström's Macroglobulinemia are produced by:

a. Granulocytes
b. Platelets
c. Lymphoplasmocytes

10) Plasmocytes are located:

a. In the bone marrow
b. In the blood
c. In the lymph nodes

11) With Waldenström's Macroglobulinemia:

a. IgM is monoclonal
b. IgM is polyclonal
c. Different IgMs are produced by plasmocytes and lymphoplasmocytes

12) The blood cell (red, white and platelets) factory is:

a. The blood
b. The bone marrow
c. The lymph nodes
d. The spinal cord

13) The bone marrow's microenvironment allows cells:

a. To multiply
b. To differentiate into red blood cells, platelets or leukocytes

14) Which propositions are true?

a. CLL lymphocytes multiply in the blood
b. Waldenström's Macroglobulinemia plasmocytes circulate in the blood
c. CLL lymphocytes are present in the bone marrow
d. CLL lymphocytes are present in the lymph nodes
e. Waldenström's Macroglobulinemia plasmocytes interfere with production of normal cells in the bone marrow

15) About the spleen:

a. The spleen is the destruction plant of aged blood cells
b. The spleen is connected to the bloodstream and lymphatic system
c. The spleen can swell with CLL
d. The spleen recycles the iron from the red blood cells

16) With CLL and Waldenström's Macroglobulinemia, karyotypes can detect:

a. Acquired abnormalities present only in diseased cells
b. If my disease is hereditary

17) The karyotype helps spot:

a. Trisomies
b. Chromosome deletions
c. Mutations
d. Chromosome translocations

18) What abnormality causes cells to resist Fludarabine?

a. 13q deletion
b. 11q deletion
c. 17p deletion

19) The genetic code:

a. Can copy DNA into RNA
b. Can copy DNA into protein
c. Can translates RNA into protein

20) How to spot a mutation?

a. With immunophenotyping
b. With a karyotype
c. With DNA sequencing

21) With CLL, what assessment is necessary before starting chemotherapy?

a. A full body scan
b. A bone marrow biopsy
c. An infection work-up
d. Liver function tests

22) Chemotherapy used in CLL and Waldenström's Macroglobulinemia:

a. Always induces hair loss
b. Can cause nausea
c. Can give a fever
d. Can make you more susceptible to infections

23) Minimal Residual disease:

a. Is evaluated on the number of CLL cells identified by immunophenotyping
b. Is evaluated on electrophoresis in Waldenström's Macroglobulinemia

24) Signaling inhibitors:

a. Are taken as pills
b. Are effective on patients resistant to chemotherapy
c. Are indicated with a TP53 mutation

25) How drugs work:

a. Chemotherapy breaks down the cells' DNA
b. The Clax displace the cells from the lymph nodes
c. Signaling inhibitors attack the apoptosis mechanism
d. Monoclonal antibodies bind to the cells to be destroyed

ANSWERS

1) Answer a. Yes, everyone has lymph nodes but they are very small and not palpable when healthy

2) Answers a, b, c, e. Platelets help with coagulation.

3) Answers a, b, c, d.

4) Answer b. No, they are CLL lymphocytes. Their membrane is more fragile and some break when blood is spread on the blade.

5) Answers a, b. The diagnosis is suspected on lymphocytes increase on the FBC and the diagnosis is confirmed with immunophenotyping.

6) Answer b. No, it's stage B. In stage C, there is either anemia or a platelet decrease, whether lymph nodes are swollen or not.

7) Answer a. Yes in all cases. This is very important because CLL makes you more susceptible to infections.

8) Answers a, b.

9) Answer c.

10) Answer a. Plasmocytes stay in the bone marrow and do not circulate.

11) Answer a.

12) Answer b. All blood cells are made in the bone marrow. Lymphocytes produced in the bone marrow up to the mature stage can then multiply in the lymph nodes.

13) Answers a, b.

14) Answers c, d, e.

15) Answers a, b, c, d.

16) Answer a. CLL and Waldenström's Macroglobulinemia karyotypes only concerns sick cells, not healthy cells. Furthermore there is no known hereditary chromosomal abnormality in these diseases.

17) Answers a, b, d. Mutations are located on the DNA sequence. They are too small to be visible on chromosomes.

18) Answer c. The 17p deletion involves a TP53 gene that is important for cells to be sensitive to chemotherapy. With the 17p deletion, the gene doesn't work and the diseased cells are unaffected by chemotherapy.

19) Answer c. RNA is an exact copy of a DNA strand coming from the nucleus. RNA will then be translated into protein thanks to the genetic code that translates each triplet of bases into an amino acid. The amino acid sequence forms the protein that corresponds to the «recipe» in the DNA.

20) Answer c. Only DNA sequencing can show mutation. Immunophenotyping identifies markers on the cells and the karyotype identifies chromosome abnormalities.

21) Answers a, c, d. The bone marrow biopsy is useless before treatment.

22) Answers b, c, d. This type of chemotherapy does not induce hair loss. It can cause nausea that is well controlled by current treatments. It does not give fever; fever is the sign of an infection that must be treated.

23) Answers a, b.

24) Answers a, b, c.

25) Answers a, d. Clax molecules (like Venetoclax) are drugs that restore the defective apoptosis of CLL. Signaling inhibitors interrupt the survival signals that the cells receive in the lymph nodes and allow them to be expelled.

Through these comic strips, we hope to shed some light on the puzzles surrounding the lymphocyte by way of an imaginary voyage through the body's fascinating internal machinery. With their simple and direct style, Nat Mikles' drawings allow us to peer into this complex world in a fun and didactic way.

We thank all of those who have enriched this project through their competence and know-how: Florence Cymbalista, Véronique Leblond, Anne-Sophie Michallet, Vincent Camus, Guillaume Cartron, Michel Goudard, Xavier Troussard and Loïc Ysebaert. Several of these experts work together within the *French Innovative Leukemia Organization (**FILO**),* which specializes in the field of leukemia research.

We also thank the ***GILEAD*** and ***JANSSEN*** pharmaceutical companies for their trust; their institutional support has allowed us to create this English edition.

If there is one ambition that we all hold dear, it is that of inviting the reader, the patient, family and relatives to know a little more, to better grasp the patient's disease and the sometimes complex path of medical tests in order to help them to discover the important progress of contemporary therapy.

Christian Puppinck
President of SILLC

The *Association for Chronic Lymphocytic Leukemia and Waldenström's Macroglobulinemia Support and Information (**SILLC**)* is open to patients, their families, relatives as well as health care professionals.

Translated from French to English by Didier Legaré-Gravel.
Rereading and corrections by Thérèse Bouchez.

Printed in Paris, October 2018.