Que reste-t-il de l'anatomie pathologique à l'heure de la biologie moléculaire?

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Un peu d'histoire

- **Description macroscopique des lésions depuis l'antiquité.**
- **Le microscope est mis au point à la fin du XVIIème siècle.**
- **Théophile Bonet publie en 1679 à Genève « *le Sepulchretum* » le premier traité d'anatomo-pathologie.**
- **X. Bichat publie en 1802 son « Traité d'anatomopathologie », les cancers ne sont plus décrits selon leur aspect clinique, mais selon l'organe et le tissu d'origine.**
- **Jean Lobstein crée à Strasbourg en 1819 la première Chaire indépendante d'anatomie pathologique.**

La place de l'anatomopathologie

Avant l'heure de la biologie moléculaire:

- Place essentielle dans le diagnostic: **il n'y a pas de diagnostic de cancer sans preuve anatomo-pathologie sauf exception.**
- Permet une classification et une sous classification des tumeurs.
- L'anatomo-pathologie donne des éléments essentiels sur le pronostic et parfois prédictifs de la thérapeutique.

Certains vont être décisionnels sur la stratégie thérapeutique:

- Ré-intervention (marges de résection)
- Radiothérapie ou curiethérapie (taille tumorale, résidu, degré d'envahissement tumoral,…)
- Chimiothérapie (grade, envahissement ganglionnaire, taux de prolifération, immuno histochimie, HER2,…)
- Hormonothérapie (récepteurs hormonaux,…)

LE TOURNANT DE LA BIOLOGIE MOLÉCULAIRE
VERS LE TRAITEMENT PERSONNALISÉ

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

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April 2.

[1] Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).
[2] Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).
[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).
[4] Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).
[5] Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).
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Molecular Structure of Deoxypentose Nucleic Acids

WHILE the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury[1]) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios

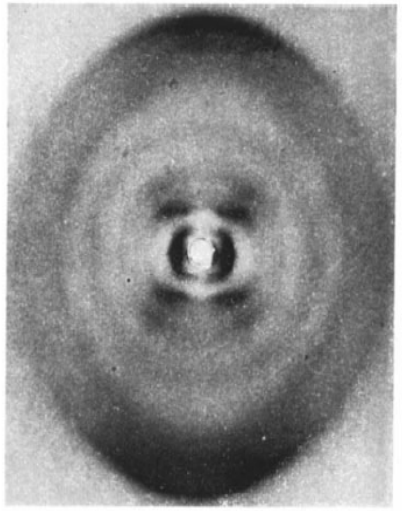


Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B. coli*. Fibre axis vertical

the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats n times along the helix there will be a meridional reflexion (J_0^2) on the nth layer line. The helical configuration produces side-bands on this fundamental frequency, the effect[5] being to reproduce the intensity distribution about the origin around the new origin, on the nth

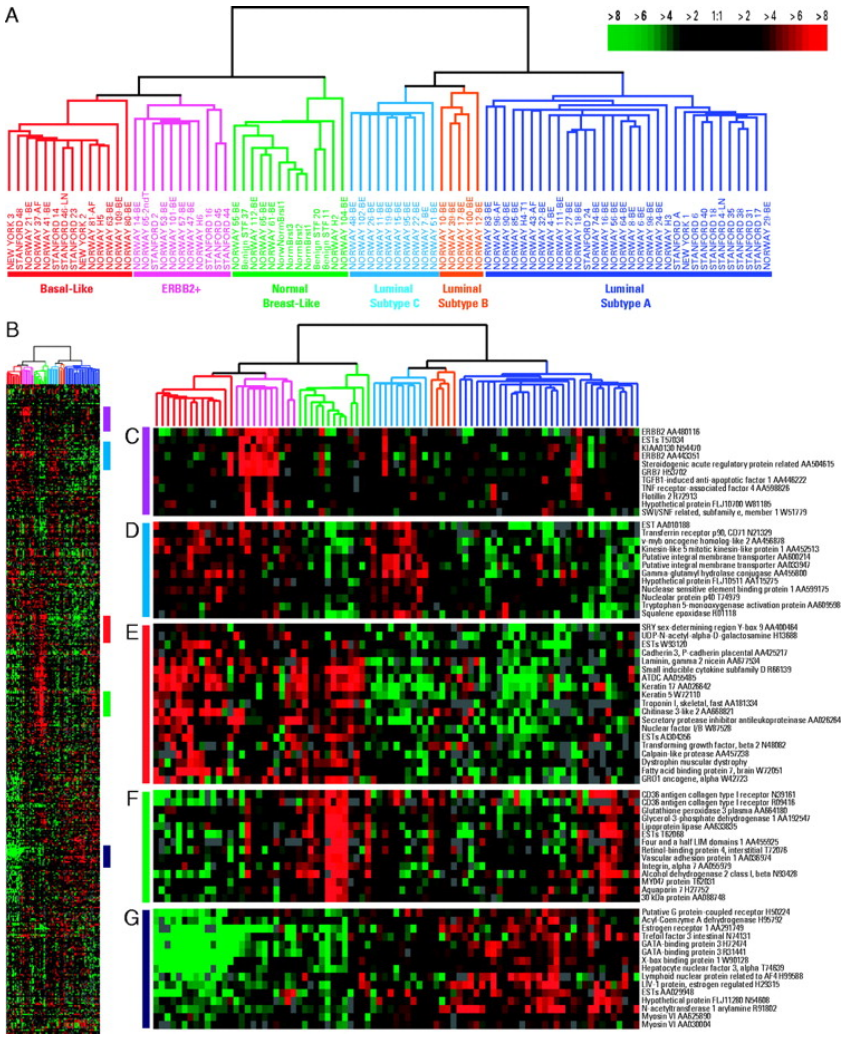
- **Une nouvelle révolution est en marche avec la découverte de la structure spatiale l'ADN par Watson et Crick.**

- **Puis la découverte par Bishop, Varmus et Stehelin des oncogènes en 1976.**

- **Une nouvelle théorie physiopathologique du cancer apparait basée sur des altérations génétiques acquises ou plus rarement germinales ouvrant la voie à des thérapies ciblées ou de précision.**

Classification moléculaire à visée pronostique et prédictive: exemple cancer du sein

456 gènes identifiant 5 classes de tumeurs différentes

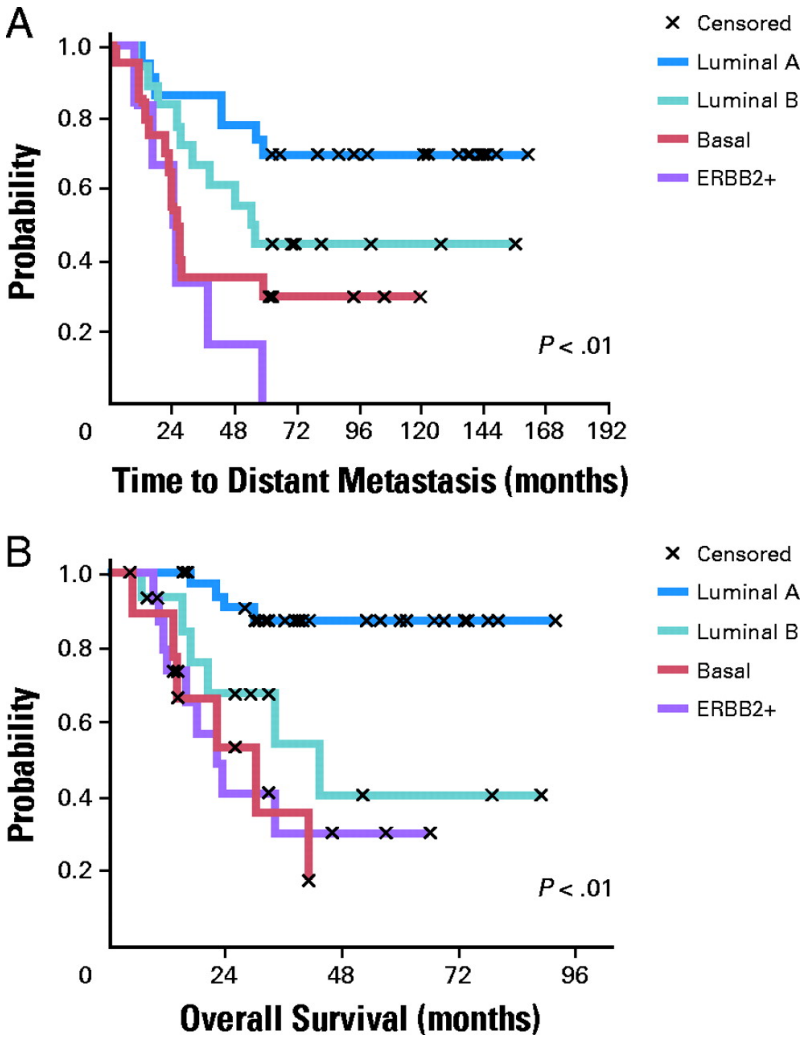


- classe proche des cel. myoépithéliales, basales
- classe proche du tissu mammaire normal
- classe surexprimant HER2

- classe proche des cel. épithéliales luminales (A,B)

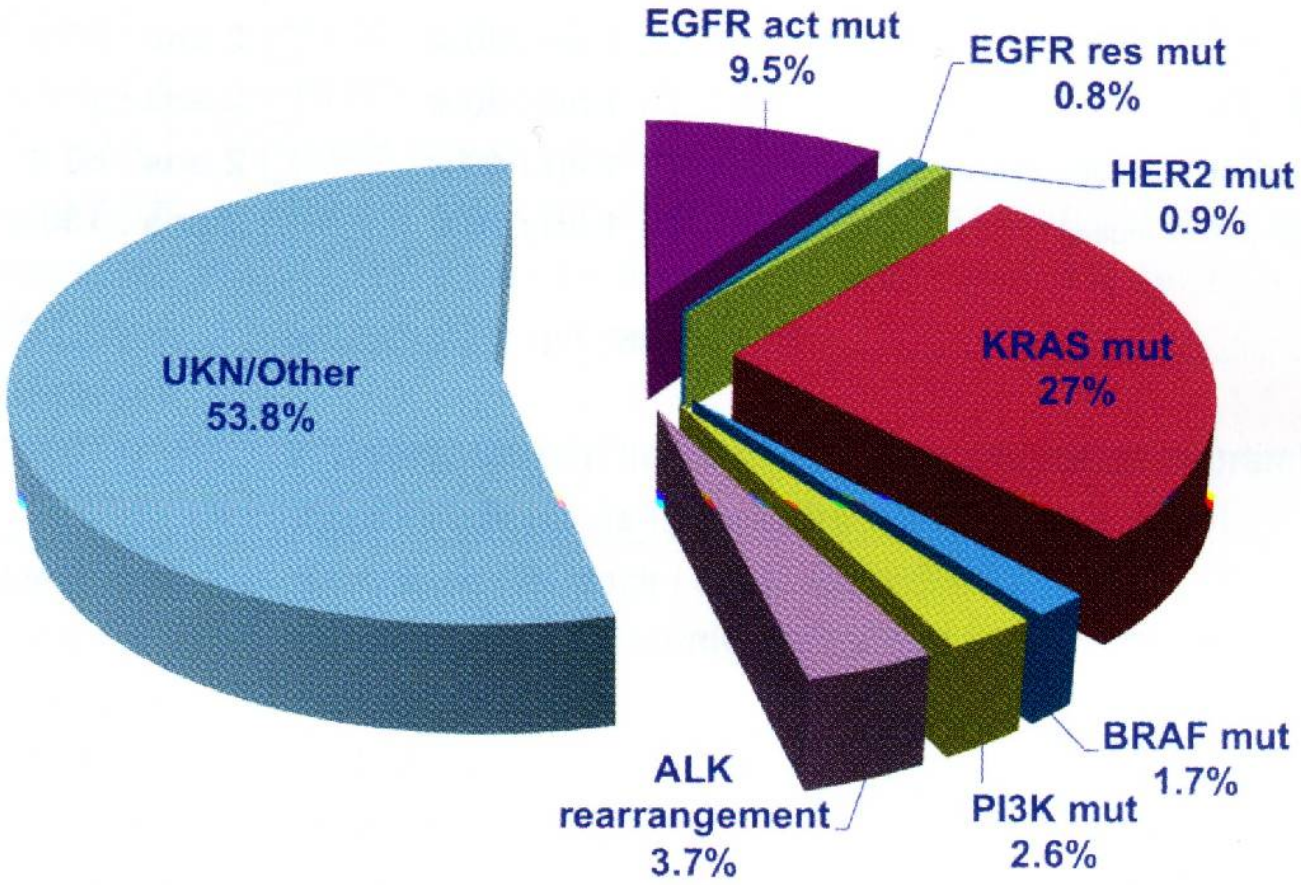
Sorlie Therese et al. (2001) Proc. Natl. Acad. Sci. USA 98, 10869-10874

Survie globale et survie en rémission selon la classification moléculaire



Brenton, J. D. et al.
J Clin Oncol; 23:7350-7360 2005

Epidémiologie moléculaire en France, données de 9911 patients.



(D'après Barlesi et coll.)

- **La classification moléculaire des cancers permet une nosologie différente:**

  - Cancers hormono-dépendants (sein, prostate, utérus)
  - Cancers exprimant une activité tyrosine kinase spécifique (leucémie myéloïde chronique, tumeurs stromales, syndrome hyperéosinophile,...)
  - Cancers exprimant un gène K-Ras muté ou non……..

- **Cette classification devient une base de la stratégie thérapeutique.**
- **Ces données de biologie moléculaire vont permettre le démembrement de nombreuses pathologies dite « indifférenciées » .**
- **Le rôle purement diagnostique de la pathologie évolue vers une aide à la définition de la stratégie thérapeutique**

Profil génomique et traitement

Exemple des tumeurs stromales (GIST) :

Exprime CD 117 qui est une partie du récepteur c-Kit et dans 80% des cas il y a une surexpression de c-Kit liée à des mutations activatrices dans le gène. Mais le type de mutation gouverne la stratégie thérapeutique.

- **Mutation c-KIT :**
  - **Exon 11 : Bon répondeur à Imatinib dose standard (400 mg/j)**
  - **Exon 9 : répondeur médiocre à des doses standards ; il faut doubler la dose (800 mg/j)**
- **Mutation PDGFRA :**
  - **Résistance à l'Imatinib si mutation dans un codon particulier D842V ; traitement avec autre TKI, traitement standard dans les autres mutations.**

Vers une classification oncobiologique ?

- **Tumeur HER-2 + (Sein, Estomac,…)**
- **Tumeur EGFR + (Poumon NPC, Colorectal, VADS,…)**
- **Tumeur VEGF (Rein, Colorectal, Sein, Ovaire,…)**

…… Mais nécessité d'un diagnostic morpho histologique de cancer donné par l'anatomo pathologiste.

La biopathologie

- **Trois partenaires qui doivent travailler ensemble:**
  - Anatomo pathologiste.
  - Biologiste moléculaire.
  - Bio informaticien.
    - Chacun doit exister pleinement pour réussir et avancer

- **Un outil indispensable à gérer ensemble:**
  - La tumorothéque

……Mais les ménages à trois ne sont pas toujours faciles!!!!

Qui fait quoi?

- **Rappel sur la prise en charge des cancers il y a 20 ans:**
  - Les chirurgiens décidaient seuls de la stratégie.
  - Les radiothérapeutes faisaient la chimiothérapie.
- **Aujourd'hui:**
  - Face à la complexité des traitements chacun a développé son propre champ d'activité.
  - Mais décision finale concertée.
  - Besoin de structures de concertation donnant une cohérence à l'approche biopathologique et à l'approche soins.

Conclusion

- **Le diagnostic histopathologique est et sera toujours indispensable.**
- **L'anatomopathologiste est garant de la qualité des prélévements qui est l'élément indispensable à des résultats de biologie moléculaire fiables.**
- **Mais nécessité d'évolution vers un nouveau compte rendu biopathologique:**

Le nouveau compte rendu

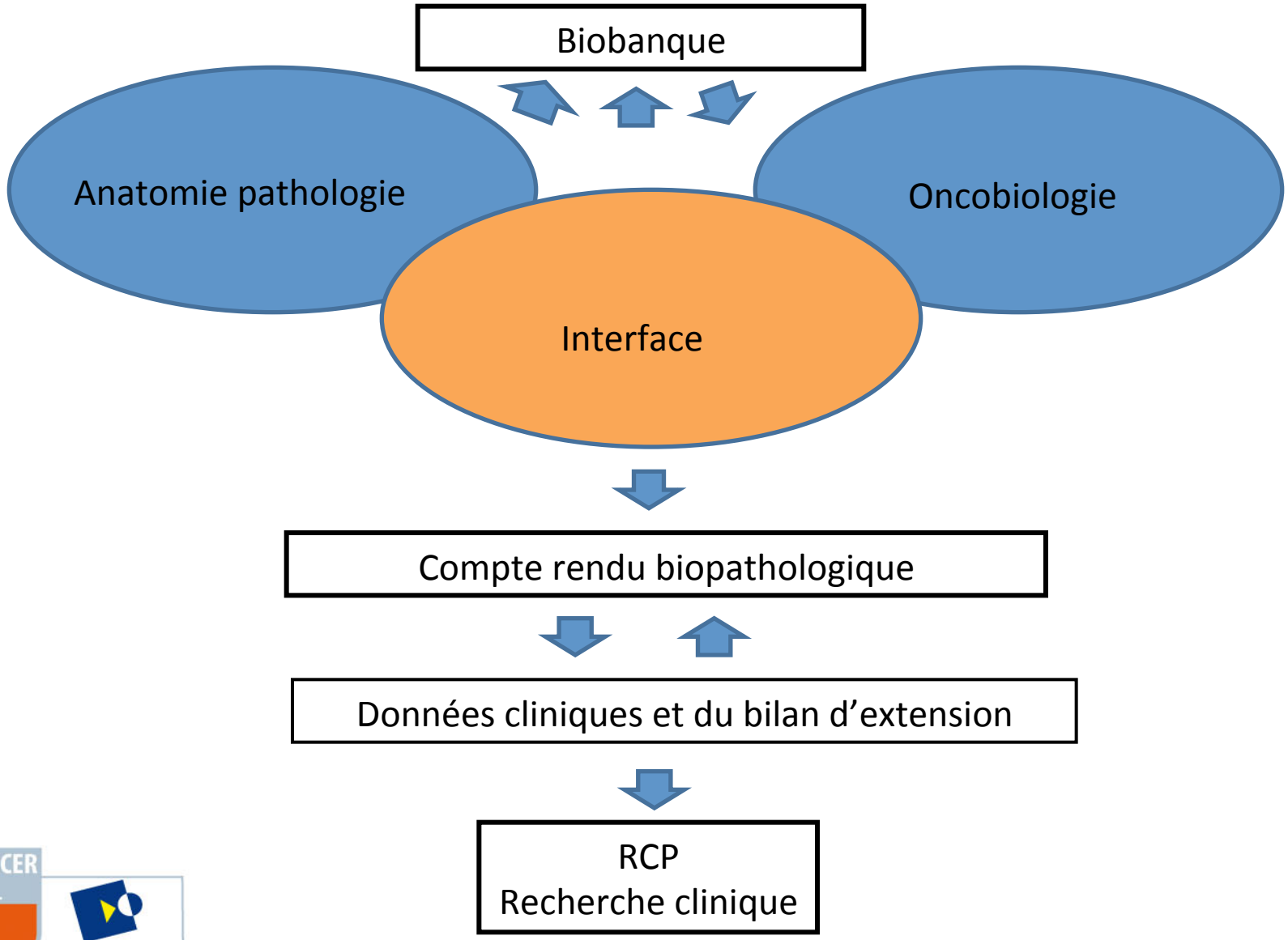
- **Données histopathologiques**

- **Classification biomoléculaire**

- **Données de biologie moléculaire sur:**
  - Cibles thérapeutiques potentielles.
  - Choix et dose des drogues potentielles en tenant compte des données des gènes impliqués dans le catabolisme des drogues.
  - Exploration des mécanismes potentielles de résistance (traitement de deuxième ligne).

- **Ce nouveau compte rendu impliquant anatomopathologiste, biologiste moléculaire, bio informaticien sera l'outil indispensable à la proposition thérapeutique de la RCP.**

- **Il sera la base de la recherche clinique et du choix du traitement médical par l'onco-pharmacien et l'oncologue.**



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