

Applications of droplet-based digital PCR approaches in cancer research

Scientists involved: Gitta Boons, Ouriel Caën, Jerome Durand-Labrunie, Fanny Garlan, Sonia Garrigou, Hélène Guermouche, Heng Lu, Solveig Meles, Roberta Menezes, Dr Philippe Nizard, Corinne Normand, Dr Daniel Pietrasz, Dr Karla Perez-Toralla, Dr Shufang Renault, Dr Eleonora Zonta

Scientists involved previously: Evelyne Liuu, Jean-François Bartolo, Zakaria El Harrak, Deniz Pekin, Thevy Hor

Droplet-based digital procedure presents high potentialities for cancer research. Consequently in parallel to the development of highly sensitive quantitative microfluidic tools for cancer biomarkers detection, part of our research is dedicated to their validation. Multiplex strategies were thus developed and validated for the analysis of DNA integrity (Didelot *et al.*, Clin. Chem. 2013) as well as the detection of the seven most frequent *KRAS* mutations within patient samples including plasma (Taly *et al.*, Clin. Chem. 2014) and tumors (Laurent-Puig *et al.* submitted).

Future projects of the team are aiming at evaluating the use of circulating tumor DNA as a potential marker for patient follow-up and investigating the role of low frequency subclones for cancer treatment. Our main focuss is directed against colorectal cancer (collab. Pr. P. Laurent-Puig) and lung cancers (collab. Dr H. Blons), we are also developing tools and strategies for other cancers through collaborative projects.

FUNDINGS

Ligue Nationale contre le cancer (Labelization), INCa (Institut National du Cancer), ARC (Association pour la recherche sur le Cancer, "Equipe à l'honneur" award, PhD fellowship) Foundation, ANR (French National Research Agency) Nanotechnologies, CARPEM (Cancer research for personalized medicine SIRIC network), Merieux Institute (Advanced Research Grant, PLP & VT), Fondation Servier (PhD fellowship), French National Alliance for Life Sciences and Health (Aviesan, PhD fellowships)

INSTITUTIONAL SUPPORT

CNRS, INSERM, Université Paris-Descartes

COLLABORATORS

Pr Pierre Laurent-Puig (UMRS1147, hEGP hospital)

Pr Hélène Blons (UMRS1147, hEGP hospital)

Pr Elisabeth Fabre (UMRS1147, hEGP hospital)

Dr Nicolas Pecuchet (UMRS1147, hEGP hospital)

Dr Aziz Zaanani (UMRS1147, hEGP hospital)

Dr Jean-Baptiste Bachet (UMRS1147, Pitié Salpêtrière Hospital)

Dr Anne-Sophie Bats (UMRS1147, hEGP hospital)

Dr Charlotte Ngo (UMRS1147, hEGP hospital)

Pr Jean Donadieu (AP-HP, Trousseau Hospital, Paris)

Dr Sebastien Héritier (AP-HP, Trousseau Hospital, Paris)

Pr Jean-François Emile (AP-HP, Ambroise Paré Hospital, Paris)

Dr Olivier Kosmider (AP-HP, Cochin Institute, Paris)

Pr Iradj Sobahni (AP-HP, UPEC, Henri-Mondor Hospital, Paris)

Pr Marc Sanson, (Brain and Spine Institute, ICM, Paris)

Dr Yannick Marie, (Brain and Spine Institute, ICM, Paris)

Pr. Paul Hofman (CHU Nice)

Pr. Elena Paillaud, Dr Evelyne Liuu (Henri Mondor Hospital, Créteil, AP-HP)

Raindance Technologies. Lexington, MA, US.

PUBLICATIONS

Multiplex detection of rare mutations by picoliter droplet based digital PCR : sensitivity and specificity

considerations. E. Zonta, F. Garlan, N. Pecuchet, K. Perez-Toralla, C. Milbury, A. Didelot, O. Caen, H. Blons, E. Fabre, P. Laurent-Puig, V. Taly*. *Plos One* (2016). [Journal Link](#).

BRAF mutation correlates with High-Risk Langerhans Cell Histiocytosis and increased resistance to first-line therapy.

S. Héritier, J-F. Emile, M. Aziz-Barkaoui, C. Thomas, S. Fraitag, S. Boudjemaa, F. Renaud, A. Moreau, M. Peuchmaur, C. Chassagne-Clément, F. Dijoud, V. Rigau, D. Moshous, A. Lambilliotte, F. Mazingue, K. Kebaili, J. Miron, E. Jeziorski, G. Plat, N. Aladjidi, A. Ferster, H. Pacquement, C. Galambrun, L. Brugières, G. Leverger, L. Mansuy, C. Paillard, A. Deville, C. Armari-Alla, A. Lutun, M. Gillibert-Yvert, J-L Stephan, F. Cohen-Aubart, J. Haroche, I. Pellier, F. Millot, B. Lescoeur, V. Gandemer, C. Bodemer, R. Lacave, Z. Hélias-Rodzewicz, V. Taly, F. Geissmann, and J. Donadieu*. *Journal of Clinical Oncology*, (2016), [Epub ahead of print]. [Pubmed](#).

A Study of Hypermethylated Circulating Tumor DNA as a Universal Colorectal Cancer Biomarker.

S. Garrigou, G. Perkins, F. Garlan, C. Normand, A. Didelot, D. Le Corre, S. Peyvandi, C. Mulot, R. Niarra, P. Aucoatourier, G. Chatellier, P. Nizard, K. Perez-Toralla, E. Zonta, C. Charpy, A. Pujals, C. Barau, O. Bouché, J.-F. Emile, D. Pezet, F. Bibeau, J. B. Hutchison, D. R. Link, A. Zaanani, P. Laurent-Puig, I. Sobhani and V. Taly*. *Clinical Chemistry* (2016), 62(8):1129-39. [Pubmed](#).

University Paris Descartes/CNRS/INSERM Press release.

InsERM Press release (english)

Droplet-based digital PCR: application in cancer research. G. Perkins, H. Lu, F. Garlan and V Taly*. *Advanced in clinical Chemistry* (2016), *in press*.

Base position error rates analysis of next generation sequencing to detect circulating tumor DNA mutations.

N. Pécuchet, Y. Rozenholc, E. Zonta, D. Pietraz, A. Didelot, J-B. Bachet, E. Fabre, V. Taly, H. Blons, P. Laurent-Puig. *Clinical Chemistry* (2016), *in press*.

Quel avenir pour l'ADN tumoral circulant? Etat des lieux et perspectives dans les cancers colorectaux, pulmonaires non à petites cellules et pancréatiques. D. Pietrasz, N. Pécuchet, E. Fabre, H. Blons, V. Taly, P. Laurent-Puig, J-B Bachet*. Bulletin du cancer (2015), 103(1):55-65. *Pubmed*.

Why and how immunohistochemistry should now be used to screen for the BRAFV600E status in metastatic melanoma? The experience of a single institution (LCEP, Nice, France). Long E, Ilie M, Lassalle S, Butori C, Poissonnet G, Washetine K, Mouroux J, Lespinet V, Lacour JP, Taly V, Laurent-Puig P, Bahadoran P, Hofman V, Hofman P.* J Eur Acad Dermatol Venereol (2015), 29(12):2436-43. *Pubmed*.

Variations of BRAF mutant allele percentage in melanomas. Hélias-Rodzewicz Z, Funck-Brentano E, Baudoux L, Jung CK, Zimmermann U, Marin C, Clerici T, Le Gall C, Peschaud F, Taly V, Saiag P, Emile JF.* BMC Cancer (2015), 15:497. *Pubmed*.

Parallelized ultra-high throughput microfluidic emulsifier for multiplex kinetic assays. Lim, J., Caen, O., Vrignon, J., Konrad, M., Taly, V. and Baret, J-C.* Co-first name for Lim, J. and Caen, O. Biomicrofluidics (2015), 9(3):034101. *Pubmed*.

Assessment of DNA integrity, applications for cancer research. Zonta, E., Nizard, P. and Taly, V. Advances in Clinical Chemistry (2015), 70:197-246. doi: 10.1016/bs.acc.2015.03.002. Epub 2015 Apr 11. Review. *Pubmed*.

Microfluidics and Tumor DNA. Contribution of digital PCR (technical review in French, french title: Microfluidique et ADN tumoral. Apport de la PCR digitale). Nizard, P., Krol, A., Laurent-Puig, P. and Taly, V. Techniques de l'ingénieur (2015). *In press*.

[Digital PCR compartmentalization I. Single-molecule detection of rare mutations]. Perez-Toralla, K., Pekin, D., Bartolo, J.-F., Garlan, F., Nizard, P., Laurent-Puig, P., Baret, J.-C. and Taly, V. Medecine/Sciences (2015), 31(1):84-92. Review. French. *Pubmed*.

[Digital PCR compartmentalization II. Contribution for the quantitative detection of circulating tumor DNA]. Caen, O., Nizard, P., Garrigou, S., Perez-Toralla, K., Zonta, E., Laurent-Puig, P. and Taly, V.* Medecine/Sciences (2015), 31(2):180-6. Review. French. *Pubmed*

Clinical relevance of KRAS-mutated sub-clones detected with picodroplet digital PCR in advanced colorectal cancer treated with anti-EGFR therapy. Laurent-Puig, P.*, Pekin, D., Normand, C., Kotsopoulos, SK, Nizard, P., Perez Toralla, K., Rowell, R., Olson, J., Srinivasan, P., Le Corre, D., Hor, T., El Harrak, Z., Li X., Link, D.R., Bouche, Emile, J-F., O., Landi, B., Boige, V., Hutchison, J.B. and Taly, V.* Clinical Cancer Research (2015), 21(5):1087-97. *Pubmed*.

Recurrent RAS and PIK3CA mutations in Erdheim-Chester disease. Emile, J-F., Diamond, E.L., Hélias-Rodzewicz, Z., Cohen-Aubart, F., Charlotte, F., Hyman, D.M., Kim, E., Rampal, R., Patel, M., Ganzel, C., Aumann, S., Faucher, G., Le Gall, C., Leroy, K., Colombat, M., Kahn, J-E., Trad, S., Nizard, P., Donadieu, J., Taly, V., Amoura, Z., Abdel-Wahab, O. and Haroche, J. Blood (2014), 124(19): 3016-9. *Pubmed*.

Association of both Langerhans cell histiocytosis and Erdheim-Chester Disease linked to BRAFV600E mutation. A multicenter study of 23 cases. B Hervier, J Haroche, L Arnaud, F Charlotte, J Donadieu, A Néel, F, Lifermann, C Villabona, B Graffin, O Hermine, A Rigolet, C Rouville, E Hachulla, T Carmoi, M Bézier, V Meignin, M Conrad, L Marie, E Kostrzewa, JM Michot, S Barete, V. Taly, K Cury, JF Emile, Z Amoura. Blood (2014), 124 (7) : 1119-1126. *Pubmed*.

Comment in

- A common progenitor cell in LCH and ECD. [Blood. 2014]

Circulating DNA, digital PCR and colorectal cancers. Taly, V and Laurent-Puig, P. Correspondances en Onco-theranostic (2013), 2(4), 184-189 (Invited review for the thematic issue : Cellules tumorales et ADN libre circulant). *Journal Link*.

Multiplex Picodroplet Digital PCR to Detect KRAS Mutations in Circulating DNA from the Plasma of Colorectal Cancer Patients. Taly V, Pekin D, Benhaim L, Kotsopoulos SK, Le Corre D, Li X, Atochin I, Link DR, Griffiths AD, Pallier K, Blons H, Bouché O, Landi B, Hutchison JB, Laurent-Puig P. Clin Chem. (2013), 59(12):1722-31. *Pubmed*.

Multiplex picoliter-droplet digital PCR for quantitative assessment of DNA integrity in clinical samples. Didelot A, Kotsopoulos SK, Lupo A, Pekin D, Li X, Atochin I, Srinivasan P, Zhong Q, Olson J, Link DR, Laurent-Puig P, Blons H, Hutchison JB, Taly V. Clinical Chemistry (2013), 59(5):815-823.

Detecting Biomarkers with microdroplet technology. Taly, V.*, Pekin, D., El Abed, A. and Laurent-Puig P. Invited Review. Trends in Molecular Medicine (2012), 18(7): 405-16. *Pubmed*.



Journal Cover.

KRAS mutation detection trap. Benhaim, L., Maley, K., Le Corre, D., Blons, H., Taly, V., Bibeau, F., Emile, J-F. and Laurent-Puig, P. Journal of Clinical Oncology (2011), Letter, 29(8): e208-e209. *Link*.

Proceedings

Picoliter droplet-based digital PCR for the quantitative and sensitive detection of rare mutations and the assessment of DNA integrity. Deniz Pekin, Audrey Didelot, Steve K. Kotsopoulos, Jean-Christophe Baret, Delphine Le Corre, Brian Hutchison, Darren R. Link, Pierre Laurent-Puig, Andrew D. Griffiths, and Valérie Taly. Proceedings of the 3rd European Conference on Microfluidics - Microfluidics 2012 - Heidelberg, December 3-5, 2012

Quantitative detection of circulating tumor DNA in plasma samples by droplet digital PCR. Pekin D., Kotsopoulos S., Xinyu L., Atochin I., Gang H., Le Corre D., Benhaim L., Hutchison J. B., Link D. R., Blons H., Laurent-Puig P. and Taly V.* The 16th International conference on miniaturized systems for Chemistry and Life Science (MicroTas 2012). Oct. 28- Nov. 1, 2012. Okinawa. Japan.

Quantitative detection of circulating tumor DNA by droplet-based digital PCR. Valerie Taly, Deniz Pekin, Steve Kotsopoulos, Xinyu Li, Ivan Atochin, Hu Gang, Delphine Le Corre, Leonor Benhaim, J. Brian Hutchison, Darren R. Link, Hélène Blons, Pierre Laurent-Puig. Cancer Research (2012), 72 (8,Supplement 1). doi: 10.1158/1538-7445.AM2012-LB-422 American Association for Cancer annual meeting (AACR 2012). March 31-April 4th, Chicago, USA.

Multiplex highly sensitive detection of cancer biomarkers in biological samples. D. Pekin, Y. Skhiri, Baret, J.-C., Le Corre, D., Mazutis, L., Ben Salem, C., El Abed, A., Hutchison, J.B., Link, D.R., Griffiths, A.D., Laurent-Puig, P. and Taly, V. In: Proceedings of the 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), 2011, p. 665-667. ISBN: 978-0-9798064-4-5.

Previous fundings

Région Alsace, Université de Strasbourg.

