



**HAL**  
open science

## The pATM Immunofluorescence assay: a high-performance radiosensitivity assay to predict post radiotherapy overreactions.

Guillaume Vogin, Thierry Bastogne, Larry Bodgi, Julien Gillet-Daubin, Aurélien Canet, Sandrine Pereira, Nicolas Foray

### ► To cite this version:

Guillaume Vogin, Thierry Bastogne, Larry Bodgi, Julien Gillet-Daubin, Aurélien Canet, et al.. The pATM Immunofluorescence assay: a high-performance radiosensitivity assay to predict post radiotherapy overreactions.. International Journal of Radiation Oncology, Biology, Physics, 2018, 101 (3), pp.690-693. 10.1016/j.ijrobp.2018.03.047 . hal-01757771

**HAL Id: hal-01757771**

**<https://hal.science/hal-01757771v1>**

Submitted on 3 Apr 2018

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# The pATM Immunofluorescence assay: a high-performance radiosensitivity assay to predict post radiotherapy overreactions

G. Vogin<sup>1,2,3</sup>, T. Bastogne<sup>4,5,6</sup>, L. Bodgi<sup>7,8</sup>, J. Gillet-Daubin<sup>7</sup>, A. Canet<sup>3,7,9,10</sup>, S. Pereira<sup>3,7</sup> and N. Foray<sup>3</sup>

<sup>1</sup>Institut de Cancérologie de Lorraine, Department of Radiation Oncology, Vandoeuvre-les-Nancy, France.

<sup>2</sup>UMR 7365 CNRS-UL, IMoPA, Vandoeuvre-les-Nancy Cedex, France

<sup>3</sup>Cancer Center of Lyon-UMR Inserm 1052 CNRS 5286 CLB, Radiobiology, Lyon, France.

<sup>4</sup>INRIA BIGS, Vandoeuvre-lès-Nancy Cedex, France,

<sup>5</sup>UMR 7039 UL-CNRS, CRAN, Vandoeuvre-lès-Nancy, France

<sup>6</sup>Cybernano, Telecom Nancy, 54602 Villers-lès-Nancy, France

<sup>7</sup>Neolys Diagnostics, R&D, Lyon, France.

<sup>8</sup>American University of Beirut Medical Center, Beirut, Lebanon

<sup>9</sup>Institut Camille Jordan-umr 5208, 21 Avenue Claude Bernard, Villeurbanne, France

<sup>10</sup>INIRIA DRACULA, 56 Boulevard Niels Bohr, Villeurbanne, France

Running title:

Updated performance of the pATM radiosensitivity assay

Corresponding author

Guillaume VOGIN, MD, PhD  
Department of radiation oncology  
Institut de Cancérologie de Lorraine  
6 avenue de bourgogne - CS 30519  
54519 Vandoeuvre-les-Nancy Cedex  
Tél : +33 3 83 59 84.27  
Fax : +33 3 83 59 83 91  
[g.vogin@nancy.unicancer.fr](mailto:g.vogin@nancy.unicancer.fr)

Author responsible for statistical analyses

Thierry BASTOGNE, PhD  
CRAN (Centre de Recherche en Automatique de Nancy)  
Département SBS  
Faculté de Médecine, Bât. D, BP 184  
54505 Vandœuvre-lès-Nancy, France  
Voice: (33) (0)3.83.68.44.73  
Fax: (33) (0)3.83.68.44.62  
[thierry.bastogne@univ-lorraine.fr](mailto:thierry.bastogne@univ-lorraine.fr)

Conflicts of interest

GV and AC have nothing to disclose.

TB is Co-Founder, Scientific Expert in Biostatistics and Nano-informatics in CYBERNANO company (Biosignal Processing & Biostatistics).

SP and LB report the following patents: FR3040178A1 FR3040179A1 WO2017098190A1 WO2017029451A1 WO2017029449A1 licensed to NEOLYS Diagnostics. SP is Head of R&D at NEOLYS Diagnostics. JGD is co-founder and CEO of NEOLYS Diagnostics.

NF reports the following patents: FR3040178A1, FR3040179A1, FR3017624A1, FR3017625A1, WO2015121597A1, WO2017098190A1, WO2017029451A1, WO2015121596A1, WO2017029449A1. NF is co-founder and Scientific Advisor of NEOLYS DIAGNOSTICS.

Authorship

GV drafted the manuscript and helped in the acquisition of data. AC and LB performed the primary statistical analyses. TB performed the secondary analyses. NF, JGD and SP interpreted the data. All authors revised the manuscript critically and have approved the final article.

Funding

This work was supported by NEOLYS Diagnostics.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29

**The pATM Immunofluorescence assay: a high-performance radiosensitivity assay to predict post radiotherapy overreactions**

Running title: Updated performance of the pATM radiosensitivity assay

**SUMMARY**

**A new statistical analysis was performed on 117 patient-specific fibroblasts lines cultured from a skin biopsy in order to assess AUC, sensitivity, specificity, the positive predicted value and the negative predicted value of the pATM Immunofluorescence assay to sort overreactors. The pATM assay shows the highest predictive performance amongst the available tests.**

**ABSTRACT**

Purpose

Identifying prior treatment, the patients who will overreact to radiotherapy (RT) would have sound positive clinical implications. By focusing on DNA double-strand breaks (DSB) recognition and repair proteins after irradiation, we recently demonstrated that the maximal number of pATM nuclear foci in the first hour (pATMmax) after ex vivo irradiation correlated with post-RT toxicity severity. We aimed to carry out additional analyses on our whole collection of fibroblast lines to refine the predictive performance of our assay.

Material and Methods

30 Immunofluorescence experiments were performed on 117 primary skin fibroblast lines irradiated at 2 Gy. The  
31 toxicity response was split up into two binary classes: 0 if toxicity grade < 2 and 1 otherwise. To assess the  
32 relationship between the quantity of pATMmax foci and toxicity grade, we applied a correlation then a supervised  
33 classification analysis. Training datasets from 13 radiosensitive patients randomly drawn with a random under-  
34 sampling technique were constituted. ROC analyses were performed with a Monte-Carlo method to estimate the  
35 optimal threshold and discriminate the responses for each data set. The discrimination cut-off was estimated as  
36 the maximum value of the  $10^4$  thresholds computed from each training subset.

### 37 Results

38 As expected, we confirmed a quasi linear dependence between toxicity and pATMmax (Fig 1A & 1B; Pearson  
39 correlation coefficient: -0.85;  $p < 2.2e^{-16}$ ). When taken as a binary predictive assay with the optimal cut-off value  
40 of 34.5 pATM foci/cell, our assay showed an outstanding predictive performance with the following values  
41 respectively for sensitivity, specificity, NPV, PPV and AUC: 1.00, 0.92, 1.00, 0.99 and 0.987.

### 42 Conclusion

43 The results of these experiments allowed us to identify pATMmax as a high performance predictive parameter of  
44 post-radiotherapy OR. Additional studies are in progress to confirm that this radiosensitivity assay reaches the  
45 same performances in any condition to adapt the clinical practice.

46

47

48

49 **MANUSCRIPT**

50

51

52

53 **Introduction**

54 Up to 15% of the patients treated with radiotherapy (RT) experience toxicity that can lead to serious sequelae and  
55 additional societal costs (1-3). In particular, their cumulative incidence at least 30 years after the diagnosis of  
56 cancer reaches 73.4% in pediatric oncology (4). Identifying these over reactors (OR) prior RT would therefore  
57 have sound positive clinical implications.

58 The severity of toxicity depends on several factors dominated by individual radiosensitivity (iRS) (5). iRS could be  
59 defined as the innate capacity of a given individual to show a particular toxicity profile to radiation - corresponding  
60 to the loss of proliferative capacity observed in vitro.

61 Since 2003, we have collected primary skin fibroblasts from patients with DNA repair deficiencies, from apparently  
62 healthy patients or from irradiated patients with various severity grades and free intervals after RT in more than 30  
63 French or Belgian hospitals. According to Baumann et al., OR are unselected patients who experienced severe  
64 side effects defined as follows, after the same standardized physical radiation dose, without evidence of  
65 treatment- or patient-related cofactors that can affect radiation tolerance (6) :

- 66 • grade  $\geq 2$  occurring the first two weeks of RT where these side effects are unexpected or  
67 • grade  $\geq 3$  lasting more than 4 weeks after the end of RT or  
68 • grade  $\geq 3$  occurring or persisting more than 90 days after the end of RT (7).

69 We measured the RT-induced distribution of candidate DNA double-strand breaks (DSB) recognition and repair  
70 proteins with an immunofluorescence assay (8). We thus recently proposed a general classification of Human iRS  
71 based on the nucleoshuttling of ATM kinase (9). However, we described a continuous distribution of the maximal  
72 number of pATM nuclear foci in the first hour (pATMmax) after ex vivo irradiation according to the 6 possible  
73 severity grades taken individually – that is not suitable to support clinical decision making (9). During ESTRO36,  
74 we reported a preliminary ROC curve analysis according to a binarized toxicity grade classification. With the  
75 optimal cut-off value of 35 foci, pATMmax assay showed promising predictive performances, with an area under  
76 the curve (AUC) of 0.97, a predictive positive value (PPV) of 99%, a specificity of 92% and a sensitivity of 100%  
77 (10).

78 Our objective in the present letter was to refine the performance of our iRS assay on the whole cohort applying a  
79 new analysis with a robust supervised classification.

80

81

82

### 83 **Material and Methods**

84

#### 85 Material description

86 This study was conducted on the 117 primary skin-untransformed fibroblast lines we previously reported including  
87 12 radioresistant, 4 ATM<sup>-/-</sup>, and 1 LIG4<sup>-/-</sup> gifted cell lines and 100 lines from OR patients (9). Among the  
88 irradiated patients in our cohort, the median time from treatment to biopsy was 13.3 months (1-273). Two  
89 independent expert radiation oncologists graded the toxicity severity according to the Common Terminology  
90 Criteria for Adverse Events (CTCAE) version 4.03. The toxicity response was split up into two binary classes: 0 if  
91 toxicity grade < 2 (“biologically radioresistant”) and 1 otherwise (“biologically radiosensitive”)

92 The regional ethical committee approved the COPERNIC collection. Cell lines were declared under the numbers  
93 DC2008-585 and DC2011-1437 to the French Ministry of Research. The database was protected under the  
94 reference as IDDN.FR.001.510017.000.D.P.2014.000.10300. All the patients were informed and gave signed  
95 consent according to the ethics recommendations.

96 The biological methods have been extensively described (8, 11).

#### 97 Statistical analysis

98 **Assessment of quality.** To assess the relationship between the quantity of pATMmax foci – defining iRS - and  
99 binarized toxicity grade, we applied a Pearson correlation test. The intra-grade variance is defined as the variance  
100 of pATMmax foci in each CTCAE group. A Student t-test and a Fisher test were carried out to examine the  
101 relevance of the affine model parameters and the adequacy between the model response and the experimental  
102 data respectively. In each test, the associated p-value indicates the compatibility of data with the null hypothesis.

103 **Supervised classification.** Our initial dataset was expected unbalanced between “radiosensitive” and  
104 “radioresistant” patients. To correct it, a random under-sampling technique was implemented, in which 13  
105 radiosensitive patients were randomly drawn from the 104 available subjects (12). As a consequence, each  
106 training dataset was first composed of 26 patients (13 radiosensitive and 13 radioresistant patients). The thirteen

107 radioresistant subjects were always the same in this study. ROC analyses were performed with a Monte-Carlo  
108 method to estimate the optimal threshold and discriminate the responses for each data set. Finally, the  
109 discrimination cut-off was estimated as the median value of the  $10^4$  thresholds computed from each training  
110 subset. The classification performances were characterized on the 117 patients by five parameters: AUC,  
111 sensitivity index, specificity index, the PPV and the negative predicted value (NPV). The analyses were carried  
112 out on R.

113

## 114 **Results**

115 Two independent expert radiation oncologists defined the severity grade in blind with total agreement.

### 116 Bivariate Analysis

117 As expected, we clearly emphasized a quasi-negative linear dependence between toxicity and pATMmax (Fig 1A;  
118 Pearson correlation coefficient: -0.85). The t-test and F-test confirmed the strong correlation between those two  
119 quantities reflecting the degradation of pATM nucleoshuttling with worsening of toxicity (p-values  $< 2.2e^{-16}$  for the  
120 two tests).

### 121 Supervised classification analysis

122 We reclassified 104 radiosensitive patients and 13 radioresistant subjects. After the Monte-Carlo session, the final  
123 classification cut-off was estimated at 34.5 pATMmax foci/cell nucleus. From the ROC curve, we obtained the  
124 following values respectively for the sensitivity, specificity, NPV, PPV and AUC: 1.00, 0.92, 1.00, 0.99 and 0.987  
125 (Fig 1B). We estimated the coefficients of variation of each quality criterion in the same order: 0, 0.019448,  
126 0.023315, 0 and 0.0076695.

127

## 128 **Discussion**

129 To our knowledge, our pATMmax predictive assay performed on skin fibroblasts show the highest performance  
130 reported so far – pointing ATM nucleoshuttling as a very good predictor for Human iRS (13-15). This quantitative  
131 correlation was found to be independent of tumor location, cell type and of early or late occurrence of the toxicity  
132 (9). Unlike other assays reporting molecular or cellular endpoints like polymorphisms or apoptosis, our assay  
133 provides: 1) a reliable prediction of radiosensitivity by considering CTCAE grades separately – and in a binarized  
134 way (9); 2) a relevant biological interpretation of the linear-quadratic model (16-18); 3) a relevant mechanistic

135 model to explain major IRS in genetic diseases associated with mutations of cytoplasm proteins interacting with  
136 pATM nucleoshuttling (11, 19, 20). Patients are currently enrolled prior RT in confirmatory prospective studies in  
137 pediatrics (NCT02827552) and sarcomas/GI malignancies (NCT02797405).

138 We are aware that the mandatory cellular amplification step after skin sampling requires several weeks that could  
139 appear not suitable for clinical use. However, we are now developing a fast ELISA-assay targeting pATM directly  
140 from the original skin sample and with still very interesting performances (average AUC of more than 0.8) and a  
141 deadline of a few days for editing the results (14).

142

### 143 Conclusion

144 When taken as a binary predictive assay, the pATMmax predictive assay appears as one of the most powerful  
145 available. Prospective studies are in progress to confirm its performance.

146

147

148

### 149 **FIGURE CAPTION**

150

151 A. Correlation analysis between the mean number of pATM foci/cell nucleus and the individual CTCAE grades.

152 Each cross denotes a radioresistant patient and each circle a radiosensitive subject. The red line represents the

153 regression providing the best fit to experimental data. B. ROC curve computed from the whole reclassified data

154 set, AUC=0.987 (CV=0.0076695)

155

156

157

### 158 **REFERENCES**

159

160

- 161 1. UNSCEAR. UNSCEAR 2008 REPORT Vol. II : EFFECTS OF IONIZING RADIATION. Publications UN,  
162 editor. Vienna: United Nations; 2008. 320 p.
- 163 2. Mohanti BK, Bansal M. Late sequelae of radiotherapy in adults. Support Care Cancer. 2005;13(10):775-  
164 80.
- 165 3. Rapport 2013 de l'Observatoire sociétal des cancers. 2014.
- 166 4. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, et al. Chronic health  
167 conditions in adult survivors of childhood cancer. The New England journal of medicine. 2006;355(15):1572-82.



168 5. Safwat A, Bentzen SM, Turesson I, Hendry JH. Deterministic rather than stochastic factors explain most  
169 of the variation in the expression of skin telangiectasia after radiotherapy. *International journal of radiation*  
170 *oncology, biology, physics*. 2002;52(1):198-204.

171 6. Bentzen SM, Overgaard J. Patient-to-Patient Variability in the Expression of Radiation-Induced Normal  
172 Tissue Injury. *Semin Radiat Oncol*. 1994;4(2):68-80.

173 7. Baumann M, Holscher T, Begg AC. Towards genetic prediction of radiation responses: ESTRO's  
174 GENEPI project. *Radiother Oncol*. 2003;69(2):121-5.

175 8. Joubert A, Zimmerman KM, Bencokova Z, Gastaldo J, Chavaudra N, Favaudon V, et al. DNA double-  
176 strand break repair defects in syndromes associated with acute radiation response: at least two different assays  
177 to predict intrinsic radiosensitivity? *International journal of radiation biology*. 2008;84(2):107-25.

178 9. Granzotto A, Benadjaoud MA, Vogin G, Devic C, Ferlazzo ML, Bodgi L, et al. Influence of Nucleoshuttling  
179 of the ATM Protein in the Healthy Tissues Response to Radiation Therapy: Toward a Molecular Classification of  
180 Human Radiosensitivity. *International journal of radiation oncology, biology, physics*. 2016;94(3):450-60.

181 10. Vogin G, Bodgi L, Canet A, Pereira S, Gillet-Daubin J, Foray N, editors. High-performance  
182 radiosensitivity assay to predict post radiation overreactions. *ESTRO 36; 2017; Vienna, Austria*.

183 11. Ferlazzo ML, Sonzogni L, Granzotto A, Bodgi L, Lartin O, Devic C, et al. Mutations of the Huntington's  
184 disease protein impact on the ATM-dependent signaling and repair pathways of the radiation-induced DNA  
185 double-strand breaks: corrective effect of statins and bisphosphonates. *Molecular neurobiology*. 2014;49(3):1200-  
186 11.

187 12. Ganganwar V. An overview of classification algorithms for imbalanced datasets. *International Journal of*  
188 *Emerging Technology and Advanced Engineering*. 2012;2(4):42-7.

189 13. Ferlazzo ML, Bourguignon M, Foray N. Functional Assays for Individual Radiosensitivity: A Critical  
190 Review. *Semin Radiat Oncol*. 2017;27(4):310-5.

191 14. Pereira S, Bodgi L, Duclos M, Canet A, Ferlazzo ML, Devic C, et al. Fast and binary assay for predicting  
192 radiosensitivity based on the nucleoshuttling of ATM protein: development, validation and performances.  
193 *International Journal of Radiation Oncology • Biology • Physics*. 2018;100(2):353-60.

194 15. Pereira S, Bodgi L, Duclos M, Canet A, Ferlazzo ML, Devic C, et al. In reply to Azria D ; The time is  
195 coming to compare radiosensitivity predictive assays by their scientific relevance and statistical performances.  
196 *International Journal of Radiation Oncology • Biology • Physics*. 2018;In Press.

197 16. Bodgi L, Canet A, Pujo-Menjouet L, Lesne A, Victor JM, Foray N. Mathematical models of radiation  
198 action on living cells: From the target theory to the modern approaches. A historical and critical review. *Journal of*  
199 *theoretical biology*. 2016;394:93-101.

200 17. Bodgi L, Foray N. The nucleo-shuttling of the ATM protein as a basis for a novel theory of radiation  
201 response: resolution of the linear-quadratic model. *Int J Radiat Biol*. 2016;92(3):117-31.

202 18. Bodgi L, Granzotto A, Devic C, Vogin G, Lesne A, Bottollier-Depois JF, et al. A single formula to describe  
203 radiation-induced protein relocalization: towards a mathematical definition of individual radiosensitivity. *Journal of*  
204 *theoretical biology*. 2013;333:135-45.

205 19. Ferlazzo ML, Foray N. Huntington Disease: A Disease of DNA Methylation or DNA Breaks? *Am J Pathol*.  
206 2016;186(7):1750-3.

207 20. Ferlazzo ML, Bach-Tobdji MKE, Djerad A, Sonzogni L, Devic C, Granzotto A, et al. Radiobiological  
208 Characterization of Tuberous Sclerosis: a Delay in the Nucleo-Shuttling of ATM May Be Responsible for  
209 Radiosensitivity. *Molecular neurobiology*. 2017.

210