



HAL
open science

Transcriptomic and immunohistochemical approaches identify HLA-G as a predictive biomarker of gestational choriocarcinoma resistance to monochemotherapy

Pierre-Adrien Bolze, Jonathan Lopez, Fabienne Allias, Touria Hajri, Sophie Patrier, Mojgan Devouassoux-Shisheboran, Jérôme Massardier, Benoit You, François Golfier, François Mallet

► To cite this version:

Pierre-Adrien Bolze, Jonathan Lopez, Fabienne Allias, Touria Hajri, Sophie Patrier, et al.. Transcriptomic and immunohistochemical approaches identify HLA-G as a predictive biomarker of gestational choriocarcinoma resistance to monochemotherapy. *Gynecologic Oncology*, 2020, 158, pp.785 - 793. 10.1016/j.ygyno.2020.05.042 . hal-03492199

HAL Id: hal-03492199

<https://hal.science/hal-03492199>

Submitted on 8 Sep 2022

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.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Transcriptomic and immunohistochemical approaches**
2 **identify HLA-G as a predictive biomarker of gestational**
3 **choriocarcinoma resistance to monochemotherapy**

4

5 Pierre-Adrien BOLZE^{1, 2, 3}

6 pierre-adrien.bolze@chu-lyon.fr

7

8 Jonathan LOPEZ⁴

9 jonathan.lopez@chu-lyon.fr

10

11 Fabienne ALLIAS⁵

12 fabienne.allias-montmayeur@chu-lyon.fr

13

14 Touria HAJRI²

15 touria.hajri@chu-lyon.fr

16

17 Sophie PATRIER⁶

18 sophie.patrier@chu-lyon.fr

19

20 Mojgan DEVOUASSOUX-SHISHEBORAN⁵

21 mojgan.devouassoux@chu-lyon.fr

22

23 Jérôme MASSARDIER⁷

24 jerome.massardier@chu-lyon.fr

25

26 Benoit YOU⁸

27 benoit.you@chu-lyon.fr

28

29 François GOLFIER^{1, 2}

30 francois.golfier@chu-lyon.fr

31

32 François MALLET³

33 francois.mallet@biomerieux.com

34

35 1. University of Lyon 1, Hospices Civils de Lyon, University Hospital Lyon Sud,

36 Department of Gynecological Surgery and Oncology, Obstetrics, 165 chemin du

37 grand Revoyet, 69495 Pierre Bénite, France

38 2. French Center for Trophoblastic Diseases, University Hospital Lyon Sud, 165 chemin

39 du grand Revoyet, 69495 Pierre Bénite, France

40 3. Joint Research Unit Hospices Civils de Lyon-bioMérieux, Hospices Civils de Lyon,

41 Lyon Sud Hospital, 165 chemin du grand Revoyet, 69495 Pierre Bénite, France;

42 Medical Diagnostic Discovery Department (MD3), bioMérieux S.A., Marcy l'Etoile,

43 France; Joint Research Unit Hospices Civils de Lyon-bioMérieux, EA 7426

44 Pathophysiology of Injury-Induced Immunosuppression, PI3, Claude Bernard Lyon 1

45 University, Edouard Herriot Hospital, Lyon, France.

46 4. University of Lyon 1, Hospices Civils de Lyon, University Hospital Lyon Sud,

47 Plateforme de Recherche de Transfert en Oncologie, Department of Biochemistry

48 and Molecular biology, 165 chemin du grand Revoyet, 69495 Pierre Bénite, France;

49 Faculté de Médecine Lyon Est, Lyon, France.; Centre de Recherche en Cancérologie

50 de Lyon, INSERM U1052, CNRS UMR5286, Lyon, France

51 5. University of Lyon 1, Hospices Civils de Lyon, University Hospital Lyon Sud,

- 52 Department of Pathology, 165 chemin du grand Revoyet, 69495 Pierre Bénite,
53 France
- 54 6. Department of Pathology, University Hospital of Rouen, F-76031 Rouen Cedex,
55 France
- 56 7. University of Lyon 1, University Hospital Femme Mere Enfant, Department of
57 Obstetrics and Gynecology, 51, boulevard Pinel, 69500 Bron, France
- 58 8. University of Lyon 1, Hospices Civils de Lyon, University Hospital Lyon Sud, Medical
59 Oncology Department, Investigational Center for Treatments in Oncology and
60 Hematology of Lyon (CITOHL), 165 chemin du grand Revoyet, 69495 Pierre Bénite,
61 France

62

63 **Corresponding author:**

64 Pierre-Adrien BOLZE, MD, PhD,

65 Associate Professor

66 French Reference Center for Trophoblastic Diseases

67 Centre Hospitalier Lyon Sud, 165 Chemin du Grand Revoyet

68 69495 Pierre Bénite, France

69 Email: pierre-adrien.bolze@chu-lyon.fr

70 Phone: +33 4 78 86 56 40

71 Fax: +33 4 78 86 65 54

72 **Short running title**

73 HLA-G and choriocarcinoma chemoresistance

74

75 **Higlights**

76 • Moles with post-curettage choriocarcinoma evolution and those with remission show
77 similar transcriptomic profiles

78 • HLA-G is under-expressed in monochemotherapy resistant gestational
79 choriocarcinoma

80 • Polychemosensitive and polychemoresistant choriocarcinoma show similar
81 transcriptomic profiles

82

83 **Word counts:**

84 Abstract: 241

85 Manuscript: 3533

86

87 **Abstract**

88 Objective: Using a transcriptional approach on tissue samples, we sought to identify
89 predictive biomarkers of post molar malignant transformation, and of choriocarcinoma
90 chemosensitivity to mono- (methotrexate or actinomycin D) or polychemotherapy
91 [EMA(Etoposide, Methotrexate, Actinomycin D)-CO(Cyclophosphamide, Vincristine) and
92 EMA-EP(Etoposide, Cisplatin)] regimens.

93 Methods: We studied the expression of a 760-gene panel (PanCancer Pathway) related to
94 oncogenesis and immune tolerance in tissue samples of complete hydatidiform moles and
95 gestational choriocarcinoma.

96 Results: We did not identify any differentially expressed gene between moles with post molar
97 malignant transformation in choriocarcinoma (n=14) and moles with remission (n=20). In
98 monochemoresistant choriocarcinoma (n=34), four genes (HLA-G, COL27A1, IL1R2 and
99 GLI3) had a significantly reduced expression and one (THEM4) had an increased expression
100 [FDR (false discovery rate) adjusted p-value ≤ 0.05] when compared to monochemosensitive
101 choriocarcinoma (n=9). The proportion of trophoblast cells and the intensity of
102 immunohistochemical HLA-G expression were reduced in monochemoresistant
103 choriocarcinoma ($p < 0.05$). In polychemoresistant choriocarcinoma (n=20) we did not identify
104 differentially expressed genes with an FDR adjusted p-value ≤ 0.05 when compared to
105 polychemosensitive choriocarcinoma (n=15). Gene pathway analysis revealed a predicted
106 activation of IFN γ in monochemoresistant choriocarcinoma and inhibited IL2 and TNF in
107 polychemoresistant choriocarcinoma. The main biological functions predicted to be altered in
108 chemoresistant choriocarcinoma were related to immunological homeostasis and
109 leukopoiesis.

110 Conclusion: HLA-G is a strong candidate gene to predict choriocarcinoma resistance to
111 monochemotherapy and that further studies are required to implement its routine
112 quantification in the decision process for the management of gestational choriocarcinoma.

113 **Introduction**

114

115 Gestational trophoblastic neoplasia (GTN) are epithelial proliferations developed from
116 placental trophoblast [1]. Although the majority of patients can be cured when treated
117 according to international standards, some of patients experience relapses and poor
118 survivals, especially those with histology of choriocarcinoma or with disseminated
119 diseases, mainly due to chemoresistance [2,3]. Except for rare GTN types such as
120 placental site trophoblastic tumors and epithelioid trophoblastic tumors, which should
121 be treated with surgical excision, the reference treatment for GTN is chemotherapy
122 [4,5]. The choice of chemotherapy regimen is based on the FIGO (Fédération
123 Internationale de Gynécologie Obstétrique) score, which is calculated with clinical,
124 biological and radiological data. A score ≤ 6 indicates a low-risk of resistance to
125 monochemotherapy, i.e. methotrexate or actinomycin D [5], while those with a score
126 ≥ 7 are treated with polychemotherapy, the most described being EMA-CO
127 (etoposide, methotrexate, actinomycin-D, cyclophosphamide and vincristine) [4-6].
128 Nevertheless, among low-risk patients, the incidence of resistance to methotrexate is
129 still 22.5-50% [7-10]. Moreover, long-term adverse effects observed with these
130 regimens are significant, with increased risks of oral carcinomas, melanoma,
131 meningioma, leukemia and early menopause after EMA-CO for example[11].

132

133 In the context of innate tolerance of trophoblast tissues and ubiquitous expression of
134 immune check-points, such as PD-L1 in GTN tissue samples, the effectiveness of
135 PD-L1 blockade with avelumab in patients resistant to methotrexate or EMA-CO is
136 being assessed in an ongoing phase II trial (NCT03135769) [12,13]. The preliminary
137 results suggested that up to 50% patients experienced disease remissions and hCG

138 normalizations after resistance to methotrexate, with only few minor side effects,
139 while the remaining patients had resistances to avelumab [14]. It raises the question
140 of the deleterious role of first-line chemotherapy that could potentially lead to a
141 depletion of the cellular anti-tumor immune response. Thus, it would be relevant to
142 predict post molar malignant transformation early, as well as the risk of resistance to
143 chemotherapy, in order to offer immunotherapy as a first-line treatment as way of
144 improving survival while avoiding additional unnecessary chemotoxicity. So far, no
145 predictive biomarkers of post molar choriocarcinoma transformation or
146 chemoresistance have been identified.

147 Using a transcriptional approach on tissue samples, the present study aimed at
148 identifying predictive biomarkers of malignant transformation of hydatidiform moles,
149 and of choriocarcinoma chemosensitivity to mono or polychemotherapy regimens.

150 **Material and methods**

151 • Patients and samples

152 Patients were registered in the French Reference Center for Trophoblastic Diseases,
153 while the study (NCT03488901) was approved by the local ethical committee. Each
154 histological diagnosis was confirmed by two referent pathologists from the Center.
155 Samples were retrieved from the French Biobank for the study of Trophoblastic
156 Diseases (CRB-HCL Hospices Civils de Lyon)[15].

157 Low-risk gestational choriocarcinoma patients (FIGO \leq 6) were treated with
158 monochemotherapy (methotrexate or actinomycin D), and high-risk patients (FIGO \geq 7
159 or resistance to monochemotherapy) were treated with polychemotherapy regimens
160 (EMA-CO or EMA-EP) between 2005 and 2017 (Table 1). Chemosensitivity was
161 defined as hCG (human chorionic gonadotropin) normalization during chemotherapy
162 including two consolidation cycles. Chemoresistance was defined as a plateau
163 (\leq 10% changes) in serum hCG concentrations, on at least four consecutive weekly
164 measurements over 3 weeks, or a rise $>$ 10% on three consecutive weekly
165 measurements over 2 weeks[16].

166 We compared the transcriptional profiles of complete hydatidiform moles
167 subsequently transformed into choriocarcinoma after curettage (n=14) to those with
168 post-curettage hCG normalization (n=20).

169 To identify predictive biomarkers of chemoresistance to mono and polychemotherapy
170 in gestational choriocarcinoma, we compared the transcriptional profiles of
171 choriocarcinoma resistant to monochemotherapy (n= 34 samples) with those
172 sensitive to monochemotherapy (n=9) (Table 2), assuming that polychemoresistant
173 choriocarcinoma were also resistant to monochemotherapy.

174 To identify predictive biomarkers of resistance to polychemotherapy, we compared
175 the transcriptional profiles of choriocarcinomas resistant to polychemotherapy (n=20),
176 with those of choriocarcinomas sensitive to polychemotherapy (n=15) (Table 1). All
177 patients registered in the French Center database were checked for available
178 choriocarcinoma tissue sample. We selected for analysis all samples with sufficient
179 amount of tissue to perform RNA extraction.

180

181 • RNA extraction

182 Macrodissection excluded peritumoral and necrotic tissue for choriocarcinoma
183 samples and endometrium for hydatidiform mole samples. RNAs were extracted from
184 Formalin Fixed Paraffin Embedded (FFPE) samples. Two to six 5- μ m slides were
185 used. Slides were first dewaxed with two baths of D-Limonene (2min) and a bath of
186 Absolut Ethanol (2min), and RNA extraction was then performed using High Pure
187 FFPE RNA Isolation Kit (Roche, Switzerland, #06483852001).

188

189 • Gene expression

190 Gene expression analysis was conducted on the NanoString nCounter gene
191 expression platform (NanoString Technologies). A mixed code set consisting of a
192 730-gene panel (PanCancer Pathway) related to 13 cancer-associated canonical
193 pathways including: MAPK, STAT, PI3K, RAS, Cell Cycle, Apoptosis, Hedgehog,
194 Wnt, DNA Damage Control, Transcriptional Regulation, Chromatin Modification, and
195 TGF- β , and a custom 30-gene panel (Supplementary table 1) selected among a T
196 cell-inflamed gene expression profile described by Ayers et al.[17] and trophoblast
197 tolerance genes was used. Depending on concentrations, hybridization with Human
198 PanCancer Progression probes (Nanostring Technologies, USA, #XT-CSO-PROG1-

199 12) was performed using 78 ng to 200ng RNA, according to manufacturer
200 instructions. After a 17-20h-incubation at 65°C, samples were processed on a
201 Nanostring nCounter FLEX platform. Raw counts for individual digital molecular
202 barcodes were normalized on 6 positive internal controls and 40 housekeeping
203 genes using nSolver 4.0 analysis software (Nanostring Technologies, USA). The
204 background was estimated from blank wells and six negative internal controls and
205 removed from raw counts.

206

207 • Generation of normalized data

208 Each sample was analyzed in a separate multiplexed reaction including each, 8
209 negative probes and 6 serial concentrations of positive control probes. Negative
210 control analysis was performed to determine the background for each sample.

211 Data was imported into nSolver analysis software (version 4.0, Nanostring
212 technologies) for quality checking and normalization of the data. A first step of
213 normalization using the internal positive controls permitted correction of potential
214 source of variation associated with the technical workflow. We calculated for each
215 sample the geometric mean of the positive probe counts. A scaling factor for a
216 sample was a ratio of the geometric mean of the sample, and the average across all
217 geometric means. For each sample, we divided all gene counts by the corresponding
218 scaling factor. Finally, to normalize for differences in RNA input we used the same
219 method as in the positive control normalization, except that geometric means were
220 calculated over 40 housekeeping genes (Supplementary Table). Results are
221 expressed in fold change induction.

222

223 Normalized counts were analyzed according to post molar malignant transformation
224 and chemoresistance profile. Expression of every single gene within the categories
225 was compared by t-test using nSolver 4.0 software. P-value and false-discovery rate
226 adjusted p-value (Benjamini-Hochberg) were computed. We identified differentially
227 expressed genes with a low stringency FDR, i.e. FDR-adjusted p-value ≤ 0.25 .

228

229 • Gene pathway analysis

230 Differentially expressed genes with low stringency (FRD<0.25) were analyzed for
231 enrichment of pathways using Ingenuity Pathway Analysis (IPA) software (Qiagen,
232 Redwood City, CA).

233

234 • Immunohistochemistry

235 Blocks sections of 4 μm were simultaneously cut, mounted, dried at 37 °C for 12 h
236 and deparaffinized with xylene and rehydrated through graded ethanol treatment.
237 Primary antibodies against p57 (57P06, 1:400, mAb, ThermoScientific), HLA-G
238 (clone 4H84, 1:50, mAb recognizing all HLA-G isoforms, Abcam), and ERBB2 (4B5,
239 1:100, mAb recognizing HER2 antigen, Ventana, Roche) were used. The
240 visualization system used was the BenchMark XT (Ventana Roche) with heat-
241 induced epitope retrieval (CC1 solution, Ventana, Roche) and the UltraView DAB
242 detection kit (Ventana, Roche).

243 Immunohistochemical staining was evaluated by one referent pathologist (FA) who
244 was blinded to the patient outcome, on an entire tissue section (6 to 7 cm²) at 100x
245 magnification using a semi-quantitative scoring system for each trophoblastic
246 subtype, i.e. cytotrophoblast, intermediate trophoblast and syncytiotrophoblast,
247 distinguished according to morphological criteria. Cytotrophoblast was characterized

248 by mononucleate cells with uniform and round nuclei, resembling the villous
249 cytotrophoblast in chorionic villi, while intermediate trophoblast was characterized by
250 large pleomorphic hyperchromatic nuclei and abundant amphophilic cytoplasm,
251 resembling the trophoblastic cells in the implantation site. The h-score score was
252 obtained after the multiplication of intensity score (0-4) and the percentage of stained
253 cells (0, 1 for 0-10%, 2 for 11-50% and 3 for $\geq 50\%$).

254 **Results**

255

256 **1. Differentially expressed gene profiles according to post molar malignant** 257 **transformation, and resistance to mono or polychemotherapy**

258 The comparison of transcriptional profiles of moles with transformation into
259 choriocarcinoma with those associated with post curettage hCG normalization
260 revealed only 5 differentially expressed genes with an extremely low FDR stringency
261 (FDR different than 1) (Table 3A). CDKN1C, a maternally expressed gene coding the
262 protein p57^{KIP2}, was the most dysregulated genes (2.74-fold, FDR=0.51) in moles
263 transformed into choriocarcinomas. Since p57^{KIP2} staining is routinely used in daily
264 pathology practice to distinguish partial and complete moles and despite an FDR
265 >0.05 , the expression of p57^{KIP2} in the complete hydatidiform mole samples of this
266 study was localized and quantified using immunohistochemistry. Quite unsurprisingly,
267 semi-quantitative assessments of p57^{KIP2} showed no significant difference in terms of
268 h-score between moles further transformed into choriocarcinoma and those with
269 remission. The expression pattern of p57^{KIP2} was restricted to the nuclei of
270 intermediate trophoblast, without any staining of villous mesenchyme, cyto or
271 syncytiotrophoblast.

272 To identify predictive biomarkers of resistance to monochemotherapy in gestational
273 choriocarcinomas, we compared the transcriptional profiles of 34 samples of
274 choriocarcinomas resistant to monochemotherapy with those of 9 samples sensitive
275 to monochemotherapy. Nineteen genes were differentially expressed between these
276 two groups with a low stringency FDR (FDR <0.25) (Table 3B). Four genes were
277 differentially expressed between these two groups with high stringency FDRs

278 (FDR<0.05): HLA-G (-9.19-fold), IL1R2 (-4.95-fold), and COL27A1 (-3.65-fold) were
279 under expressed, while THEM4 was slightly over expressed (1.55-fold) in
280 chemoresistant choriocarcinomas. GLI3 was under expressed (-1.59-fold) with an
281 FDR of 0.05 in chemoresistant choriocarcinomas.

282 To identify predictive biomarkers of resistance to polychemotherapy in gestational
283 choriocarcinoma, we compared the transcriptional profiles of 20 samples of
284 choriocarcinomas resistant to polychemotherapy with those of 15 samples sensitive
285 to polychemotherapy. Only 12 genes were differentially expressed with low
286 stringency FDRs (FDR<0.25) between these two groups (Table 3C). The most
287 differentially expressed genes with FDRs <0.1 were SOS2 (-1,61-fold), ERBB2 (-
288 2,44-fold), ITGA6 (1,79-fold), LIFR (-2,47-fold), and GADD45G (-2,75-fold). Overall, 4
289 differentially expressed genes were shared between choriocarcinomas resistant to
290 mono and polychemotherapy: COL27A1, SOS2, IL2RB, and ITGA6.

291 **2. Protein expression assessment of the most differentially expressed** 292 **genes**

293 We identified HLA-G as the most differentially expressed gene in choriocarcinomas
294 resistant to monochemotherapy. In polychemotherapy resistant choriocarcinomas,
295 ERBB2 was one of the highly differentially expressed gene, also known to be
296 involved in breast cancer tumorigenesis. As a consequence, HLA-G and ERBB2
297 protein expressions were assessed on FFPE tissue samples.

298 The expression of HLA-G protein was semi-quantified and localized using
299 immunohistochemical staining in the 43 choriocarcinoma samples selected for
300 prediction of chemoresistance to single agent regimen. HLA-G protein was detected
301 in all trophoblast subtypes of choriocarcinoma, mainly in intermediate trophoblast (IT)

302 and cytotrophoblast (CT), and weaker in syncytiotrophoblast (ST) (Figure 1A).
303 Expression pattern was homogeneous among samples and characterized by staining
304 in the cytoplasm and at the cytoplasmic membrane. The HLA-G total h-score, i.e. the
305 addition of IT, CT and ST h-scores, was significantly lower in monochemotherapy
306 resistant compared to sensitive choriocarcinomas ($p=0.03$). In the CT of
307 monochemotherapy resistant choriocarcinomas, the h-score was lower than in
308 sensitive CT ($p=0.02$) (Figure 1B).

309 In the 35 choriocarcinoma samples selected for chemoresistance prediction to
310 polychemotherapy, ERBB2 protein expression was semi-quantified and localized
311 using immunohistochemistry. ERBB2 was detected on the cytoplasmic membrane of
312 all trophoblast subtypes (Figure 1C). The ST h-score was significantly lower in
313 choriocarcinomas resistant than in those sensitive to polychemotherapy ($p=0.03$) but
314 this difference was not statistically significant for IT, CT or total h-score (Figure 1D).

315 **3. Gene network analysis**

316 The differentially expressed genes were analyzed with IPA, according to resistance
317 status of choriocarcinomas to mono or polychemotherapy. To maintain a diversity
318 compatible with a comprehensive approach of involved mechanisms, a low
319 stringency FDR threshold ($FDR<0.25$) was used. We studied the predicted biological
320 functions affected by these differentially expressed genes and their predicted
321 upstream regulators.

322 The five main biological functions predicted to be affected in resistance to mono and
323 polychemotherapy are presented in Figure 2A. Despite a wide oncogenesis-focused
324 studied gene panel, the functions mainly involved were immunological homeostasis
325 and leukopoiesis in chemoresistant choriocarcinomas. Leukopoiesis and cell

326 migration functions were shared in choriocarcinomas resistant to mono or
327 polychemotherapy. The other functions were mainly related to T cell differentiation in
328 choriocarcinomas resistant to monochemotherapy, and cell proliferation in
329 choriocarcinomas resistant to polychemotherapy.

330 The gene expression profiles identified IFN γ , IL2, TNF and IL4 as predicted
331 upstream regulators of resistance to chemotherapy, IFN γ on an activated status, and
332 IL2, TNF and IL4 on an inhibition status (Figure 2B). The gene expression profiles of
333 choriocarcinomas resistant to polychemotherapy were associated with 4 main
334 upstream regulators: IL2, TNF, beta-estradiol and TP53, each of them on an
335 inhibition status (Figure 2B). Comparative analyses suggest that resistances to mono
336 and polychemotherapy would be mainly associated with an inhibition status of
337 upstream regulators. IL2 and TNF are shared predicted upstream regulators of
338 resistance to mono and polychemotherapy, both regulated on inhibition status, while
339 INF γ is the only predicted active upstream regulator.

340 **Discussion**

341

342 Regarding post molar malignant transformation, the present study could not identify
343 any gene expression differences in between those becoming choriocarcinoma and
344 those normalizing after curettage, despite a large number of samples (34 samples), a
345 wide gene panel (770 genes), and an homogeneous definition of post molar
346 malignant transformation, i.e. the pathological diagnosis of post molar
347 choriocarcinoma.

348 Of note, we conducted a parallel study using a few qualified samples (2 FFPE paired
349 tissue samples, placental controls GEO GSE6573, GSE9984, GSE12767) and
350 GeneChip™ Human Genome U133 Plus 2.0 Array (analysis of about 47,000
351 transcripts) with consistent outcomes. No predictive value was found with 45
352 identified candidate genes (Supplementary Table 2) regarding post molar malignant
353 transformation on fresh frozen hydatidiform mole samples (16 paired tissue samples)
354 (Supplementary Figure). Note however that our best candidate gene was the
355 MALAT1 (identified by a dozen probesets) non-coding RNA described in lung, liver,
356 breast and colon cancer, and seemingly involved in the control of cell
357 proliferation[18]. The lack of significant identification of differentially expressed genes
358 with regard to tumor transformation can be interpreted by the imprecision of the
359 biological definition of the post molar malignant transformation, as by a marked
360 interindividual variability. Altogether, we can conclude that the interindividual
361 variability in transcriptional profile is remarkably high in hydatidiform mole tissue
362 samples.

363

364 Nevertheless, despite this high variability, we were able to identify a low number of
365 differentially expressed genes was found in choriocarcinomas associated with
366 resistance to mono or polychemotherapy compared to those associated with
367 chemosensitive tumors. Four genes were associated with resistance to
368 monochemotherapy with FDRs <0.05 , and to polychemotherapy with FDRs ≤ 0.08 .
369 Three of these genes, HLA-G, IL1R2 and THEM4, were specific of the resistance to
370 monochemotherapy, while two of them, ERBB2 and LIFR, appeared specific of the
371 polychemotherapy resistance.

372 In addition to our study, few transcriptomic approaches have been described in the
373 literature to attempt to answer the question of predicting chemoresistance in
374 trophoblastic tumors. The Wnt / β -catenin and PI3K / AKT / mTOR oncogenesis
375 pathways are activated in methotrexate-resistant choriocarcinoma and colorectal
376 cancer lines, related to the overexpression of the long noncoding RNA H19[19,20].
377 Despite the exhaustive presence of the genes of these two pathways in the panel of
378 genes studied, we did not observe any activation of these genes. Note however that
379 the THEM4 and PDGFA genes overexpressed in the methotrexate resistance
380 condition are associated with the PI3K / AKT pathway, and that the SFRP2 gene
381 overexpressed under the resistance to polychemotherapy condition is associated
382 with the Wnt / β -catenin pathway.

383

384 Among the differentially expressed genes, the expression pattern of the HLA-G
385 protein which was significantly reduced in choriocarcinomas resistant to
386 monochemotherapy is of interest. HLA-G is suspected to be involved in inhibition of
387 cytotoxicity[21], chemotaxis[22], or interferon gamma (IFN γ) regulation[23]. In
388 gestational trophoblastic diseases, the expression of HLA-G has been described in

389 intermediate trophoblast of gestational choriocarcinomas[24,25], and in extra-villous
390 trophoblast and decidual macrophages of hydatidiform moles[26,27]. The current
391 observation of HLA-G expression in trophoblast subtypes other than intermediate
392 trophoblast, i.e. cytotrophoblast and syncytiotrophoblast, has never reported before.
393 This observation suggests that the potential role of the sustained expression of HLA-
394 G protein as a predictive biomarker of chemosensitivity in gestational
395 choriocarcinoma has never been investigated. In line with our observations, it was
396 recently proposed that the non-response to anti PD-1 treatment (pembrolizumab)
397 was associated with a loss of HLA-G expression in choriocarcinoma trophoblast[28].
398 Beyond variations of HLA-G localization, quantitative changes of HLA-G were shown
399 in different tumor types. HLA-G expression was reported in many solid cancers[29]
400 such as esophageal, liver and colorectal cancers[30,31], and high proportions
401 (>55%) of tumor cells expressing HLA-G were associated with worse overall
402 survivals, although the involved mechanisms remained unclear[32]. In ovarian
403 carcinoma, only two studies were published, and they are concordant with our
404 observations. Rutten et al. showed that the overall survival of patients with high
405 grade ovarian carcinoma was improved when tissue HLA-G mRNA level was
406 increased compared to normal expression level[33]. In line with our observation,
407 Davidson et al. reported that the presence of HLA-G-positive tumor cells in malignant
408 effusions of ovarian carcinoma, when measured by immunohistochemistry with the
409 same 4H84 monoclonal antibody used in the present study, obtained prior to the
410 institution of chemotherapy correlated with better overall survival. This correlation
411 with an improved survival might be related to preferential chemosensitivity of HLA-G-
412 expressing cells[34]. In summary, opposed prognostics are reported according to the
413 observed HLA-G form (intracytoplasmic, membranous, free soluble or in micro-

414 vesicle) [35]. Therefore, further immunohistochemistry studies should distinguish the
415 biomarker value of these different HLA-G forms in choriocarcinoma. The HLA-G
416 staining results of the present study might then be completed with dedicated
417 monoclonal antibody such as 5A6G7, which specifically recognizes the soluble form
418 of HLA-G to define whether both membrane-bound and soluble HLA-G or either one
419 are down-regulated in chemoresistant choriocarcinomas[36]. Beyond the function of
420 HLA-G in the chemoresistance of choriocarcinoma, its regulation mechanisms also
421 remain to be elucidated. Of note, we report a minor reduction of GLI3 expression, a
422 signal transducer of the Hedgehog pathway, by monochemoresistant
423 choriocarcinomas, while Deschaseaux et al. previously demonstrated that HLA-G
424 down regulation during the maturation of osteoblast was due to the binding of GLI3 to
425 the GLI binding element within the HLA-G promoter[37]. Further investigations to
426 elucidate the interaction between HLA-G and GLI3 in the context of cancer are then
427 required.

428

429 Concerning predicted upstream regulators to the transcriptional profile observed in
430 monochemotherapy resistant choriocarcinomas, it is interesting to consider that it
431 may be related to an activated state of IFN γ in the intra or peri-tumoral area. It
432 should be noted that the CREB3L4 and HIST1H3G genes expressed in the
433 methotrexate resistance condition are associated with the type 1 interferon signaling
434 pathway. Consistently with a more tolerogenic environment, an *in vitro* study showed
435 that the treatment of JEG-3 choriocarcinoma cell line by IFN γ favored the
436 differentiation of CD4⁺HLA-G⁺ T cells. However, JEG-3 exposition to IFN γ led to an
437 increased expression of HLA-G, while in our study, an activated upstream IFN γ was
438 predicted to be associated with a downregulated HLA-G expression on

439 choriocarcinoma trophoblast[38]. Interestingly, Elias et al. showed that methotrexate
440 resistant JEG-3 choriocarcinoma cell lines exhibited upregulation of another
441 interferon type response signature, i.e. type I interferon. Moreover naïve JEG-3,
442 exposure to recombinant IFNA2 partially protected JEG-3 from the effects of
443 methotrexate[39].

444

445 Concerning polychemotherapy resistance, we identified four genes shared with
446 monochemotherapy resistant choriocarcinomas: COL27A1, IL2RB, ITGA6, and
447 SOS2. Cell proliferation and migration are the main altered biological functions
448 associated with resistance to polychemotherapy, the latter being also associated with
449 resistance to monochemotherapy. Among predicted upstream regulators to the
450 observed transcriptional profile, the down regulation of IL2 and TNF is also shared
451 with choriocarcinomas resistant to monochemotherapy.

452 We investigated protein expression and immunostaining intensity of ERBB2, one of
453 the most differentially expressed, but we were not able to associate any change with
454 resistance to polychemotherapy. ERBB2 is a member of the human epidermal
455 growth factor receptor family, and regulates the proliferation and differentiation of
456 various tumor cells[40]. Overexpression of ERBB2 defines an aggressive subtype of
457 breast cancers[41]. ERBB2 gene amplification has also been evidenced in normal
458 differentiated HLA-G+ trophoblast, thereby suggesting that copy number alterations
459 represent a normal feature of developmental processes[42]. The expression of
460 ERBB2 was reported in hydatidiform moles with contradictory conclusions on its
461 predictive value for post molar malignant transformation[43-46]. Moreover, two
462 studies reported a high variability in ERBB2 immunostaining intensity in gestational
463 choriocarcinoma, but no correlation to chemoresistance was found [43,47].

464

465 To conclude, this transcriptomic study did not reveal any predictive factors of post
466 molar malignant transformation into choriocarcinoma but identified reduced HLA-G
467 expression at both mRNA and protein levels as a potential predictive biomarker of
468 monochemotherapy resistance of gestational choriocarcinomas. This assumption
469 should be confirmed on a distinct prospective cohort, as a way of identifying patients
470 with monochemotherapy resistance early. Such a parameter could be integrated in
471 decision algorithms, currently based on FIGO score. We envision that upfront
472 treatment might be personalized in patients with decreased HLA-G expression with
473 innovative treatments, such as immunotherapy alone or in combo with methotrexate.
474 It would increase treatment efficacy and reduce useless exposition to toxic
475 polychemotherapy. To simplify HLA-G expression assessment, avoiding uterine
476 biopsies, further research on soluble or circulating forms of HLA-G, i.e. on exosomes
477 or circulating trophoblastic cells, might represent promising future diagnostic
478 methods.

479

480

481

482 **Acknowledgements.**

483 They authors would like to thank Garance Tondeur, Eliezer Aimontche and Brigitte
484 Bancel for their technical support, and would also like to acknowledge la Fondation
485 HCL – Lauréat Jeune Chercheur, the French Ligue Nationale contre le Cancer and
486 the Institut National du Cancer (INCa), which have recognized the French Center for
487 Trophoblastic Diseases as a Rare Tumor Center since 2009 and which renewed
488 funding that enabled this study

489 **Disclosure/conflict of interest**

490 The authors report no conflict of interest.

491

492 **Author contributions**

493 Drs Bolze and Mallet had full access to all the data in the study and take responsibility for the integrity
494 of the data and the accuracy of the data analysis.

495

496 Study concept and design: Bolze, Lopez, Mallet

497 Acquisition, analysis, or interpretation of data: All authors

498 Drafting of the manuscript: Bolze, Mallet

499 Critical revision of the manuscript for important intellectual content: All authors

500 Statistical analysis: Bolze, Hajri, Lopez

501 Obtained funding: Bolze

502 Administrative, technical, or material support: Patrier, Hajri, Allias, Devouassoux-Shisheboran

503 Study supervision: Bolze, Golfier, You, Mallet

504

505 **References**

506

507 [1] P. Hui, R. Baergen, A. Cheung, M. Fukunaga, D. Gersell, J.M. Lage, et al., Gestational
508 trophoblastic neoplasms, in: R.J. Kurman, C.M. L, H.G. S, Y.R. H (Eds.), WHO
509 Classification of Tumours of Female Reproductive Organs, Lyon, 2014: pp. 158–167.

510 [2] P.-A. Bolze, C. Riedl, J. Massardier, J.-P. Lotz, B. You, A.-M. Schott, et al., Mortality rate of
511 gestational trophoblastic neoplasia with a FIGO score of ≥ 13 , Am J Obstet Gynecol. 214
512 (2016) 390.e1–8. doi:10.1016/j.ajog.2015.09.083.

- 513 [3] A.E. Strohl, J.R. Lurain, Postmolar choriocarcinoma: An independent risk factor for
514 chemotherapy resistance in low-risk gestational trophoblastic neoplasia, *Gynecol. Oncol.*
515 141 (2016) 276–280. doi:10.1016/j.ygyno.2016.02.014.
- 516 [4] M.J. Seckl, N.J. Sebire, R.S. Berkowitz, Gestational trophoblastic disease, *Lancet.* 376
517 (2010) 717–729.
- 518 [5] H.Y.S. Ngan, M.J. Seckl, R.S. Berkowitz, Y. Xiang, F. Golfier, P.K. Sekharan, et al., Update
519 on the diagnosis and management of gestational trophoblastic disease, *Int J Gynaecol*
520 *Obstet.* 143 Suppl 2 (2018) 79–85. doi:10.1002/ijgo.12615.
- 521 [6] J.R. Lurain, Gestational trophoblastic disease II: classification and management of
522 gestational trophoblastic neoplasia, *Am J Obstet Gynecol.* 204 (2011) 11–18.
523 doi:10.1016/j.ajog.2010.06.072.
- 524 [7] G.E. Chalouhi, F. Golfier, P. Soignon, J. Massardier, J.-P. Guastalla, V. Trillet-Lenoir, et al.,
525 Methotrexate for 2000 FIGO low-risk gestational trophoblastic neoplasia patients: efficacy
526 and toxicity, *Am J Obstet Gynecol.* 200 (2009) 643.e1–643.e6.
527 doi:10.1016/j.ajog.2009.03.011.
- 528 [8] W.B. Growdon, A.J. Wolfberg, D.P. Goldstein, C.M. Feltmate, M.E. Chinchilla, E.S.
529 Lieberman, et al., Evaluating methotrexate treatment in patients with low-risk postmolar
530 gestational trophoblastic neoplasia, *Gynecol. Oncol.* 112 (2009) 353–357.
531 doi:10.1016/j.ygyno.2008.11.003.
- 532 [9] F. Taylor, D. Short, M.C. Winter, J. TIDY, P.M. Savage, N. Sarwar, et al., A retrospective
533 study to evaluate single agent methotrexate treatment in low risk gestational
534 choriocarcinoma in the United Kingdom, *Gynecol. Oncol.* 136 (2015) 258–263.
535 doi:10.1016/j.ygyno.2014.12.024.
- 536 [10] H. Matsui, K. Suzuka, K. Yamazawa, N. Tanaka, A. Mitsuhashi, K. Seki, et al., Relapse
537 rate of patients with low-risk gestational trophoblastic tumor initially treated with single-
538 agent chemotherapy, *Gynecol. Oncol.* 96 (2005) 616–620.
539 doi:10.1016/j.ygyno.2004.11.011.
- 540 [11] P. Savage, R. Cooke, J. O'Nions, J. Krell, A. Kwan, M. Camarata, et al., Effects of single-
541 agent and combination chemotherapy for gestational trophoblastic tumors on risks of

- 542 second malignancy and early menopause, *J. Clin. Oncol.* 33 (2015) 472–478.
543 doi:10.1200/JCO.2014.57.5332.
- 544 [12] P.-A. Bolze, S. Patrier, J. Massardier, T. Hajri, F. Abbas, A.-M. Schott, et al., PD-L1
545 Expression in Premalignant and Malignant Trophoblasts From Gestational Trophoblastic
546 Diseases Is Ubiquitous and Independent of Clinical Outcomes, *Int. J. Gynecol. Cancer.* 27
547 (2017) 554–561. doi:10.1097/IGC.0000000000000892.
- 548 [13] E. Veras, R.J. Kurman, T.-L. Wang, I.-M. Shih, PD-L1 Expression in Human Placentas and
549 Gestational Trophoblastic Diseases, *Int. J. Gynecol. Pathol.* 36 (2017) 146–153.
550 doi:10.1097/PGP.0000000000000305.
- 551 [14] L. Boissel, M. Betancur-Boissel, W. Lu, D.S. Krause, R.A. Van Etten, W.S. Wels, et al.,
552 Retargeting NK-92 cells by means of CD19- and CD20-specific chimeric antigen receptors
553 compares favorably with antibody-dependent cellular cytotoxicity, *Oncoimmunology.* 2
554 (2013) e26527. doi:10.4161/onci.26527.
- 555 [15] P.A. Bolze, J. Massardier, A. Buénerd, F. Thivolet Béjui, C. Perrin, I. Rouvet, et al.,
556 [Elaboration of a national biobank for the study of gestational trophoblastic diseases],
557 *Journal De Gynécologie Obstétrique Et Biologie De La Reproduction.* 45 (2016) 559–562.
558 doi:10.1016/j.jgyn.2015.07.006.
- 559 [16] I.A. McNeish, S. Strickland, L. Holden, G.J.S. Rustin, M. Foskett, M.J. Seckl, et al., Low-
560 Risk Persistent Gestational Trophoblastic Disease: Outcome After Initial Treatment With
561 Low-Dose Methotrexate and Folinic Acid From 1992 to 2000, *Journal of Clinical Oncology.*
562 20 (2002) 1838–1844. doi:10.1200/JCO.2002.07.166.
- 563 [17] M. Ayers, J. Lunceford, M. Nebozhyn, E. Murphy, A. Loboda, D.R. Kaufman, et al., IFN- γ -
564 related mRNA profile predicts clinical response to PD-1 blockade, *J Clin Invest.* 127 (2017)
565 2930–2940. doi:10.1172/JCI91190.
- 566 [18] M. Huarte, The emerging role of lncRNAs in cancer, *Nat. Med.* 21 (2015) 1253–1261.
567 doi:10.1038/nm.3981.
- 568 [19] K.-F. Wu, W.-C. Liang, L. Feng, J.-X. Pang, M.M.-Y. Waye, J.-F. Zhang, et al., H19
569 mediates methotrexate resistance in colorectal cancer through activating Wnt/ β -catenin
570 pathway, *Exp. Cell Res.* 350 (2017) 312–317. doi:10.1016/j.yexcr.2016.12.003.

- 571 [20] S. Yu, C. Wu, Q. Tan, H. Liu, Long noncoding RNA H19 promotes chemotherapy
572 resistance in choriocarcinoma cells, *J. Cell. Biochem.* 120 (2019) 15131–15144.
573 doi:10.1002/jcb.28775.
- 574 [21] N. Rouas-Freiss, R.E. Marchal, M. Kirszenbaum, J. Dausset, E.D. Carosella, The alpha1
575 domain of HLA-G1 and HLA-G2 inhibits cytotoxicity induced by natural killer cells: is HLA-G
576 the public ligand for natural killer cell inhibitory receptors? *Proceedings of the National
577 Academy of Sciences.* 94 (1997) 5249–5254.
- 578 [22] F. Morandi, E. Ferretti, R. Castriconi, A. Dondero, A. Petretto, C. Bottino, et al., Soluble
579 HLA-G dampens CD94/NKG2A expression and function and differentially modulates
580 chemotaxis and cytokine and chemokine secretion in CD56bright and CD56dim NK cells,
581 *Blood.* 118 (2011) 5840–5850. doi:10.1182/blood-2011-05-352393.
- 582 [23] B. Favier, J. LeMaout, E. Lesport, E.D. Carosella, ILT2/HLA-G interaction impairs NK-cell
583 functions through the inhibition of the late but not the early events of the NK-cell activating
584 synapse, *Faseb J.* 24 (2010) 689–699. doi:10.1096/fj.09-135194.
- 585 [24] G. Singer, R.J. Kurman, M.T. McMaster, I.-M. Shih, HLA-G immunoreactivity is specific for
586 intermediate trophoblast in gestational trophoblastic disease and can serve as a useful
587 marker in differential diagnosis, *Am. J. Surg. Pathol.* 26 (2002) 914–920.
588 doi:10.1097/01.PAS.0000018100.32686.43.
- 589 [25] T.-L. Mao, R.J. Kurman, C.-C. Huang, M.-C. Lin, I.-M. Shih, Immunohistochemistry of
590 choriocarcinoma: an aid in differential diagnosis and in elucidating pathogenesis, *Am. J.
591 Surg. Pathol.* 31 (2007) 1726–1732. doi:10.1097/PAS.0b013e318058a529.
- 592 [26] P. Basta, K. Galazka, P. Mach, W. Jozwicki, M. Walentowicz, L. Wicherek, The
593 immunohistochemical analysis of RCAS1, HLA-G, and B7H4-positive macrophages in
594 partial and complete hydatidiform mole in both applied therapeutic surgery and surgery
595 followed by chemotherapy, *American Journal of Reproductive Immunology.* 65 (2011) 164–
596 172. doi:10.1111/j.1600-0897.2010.00897.x.
- 597 [27] M. Rabreau, N. Rouas-Freiss, M. Landi, C. Le Danff, E.D. Carosella, HLA-G expression in
598 trophoblast cells is independent of embryonic development, *Hum. Immunol.* 61 (2000)
599 1108–1112.

- 600 [28] M.J. Seckl, E. Ghorani, Pembrolizumab induces long term complete remission of
601 chemotherapy resistant gestational trophoblastic neoplasia, in: XIX World Congress
602 Gestational Trophoblastic Diseases, Amsterdam, The Netherlands, 2017.
- 603 [29] N. Rouas-Freiss, P. Moreau, J. LeMaout, E.D. Carosella, The dual role of HLA-G in
604 cancer, *Journal of Immunology Research*. 2014 (2014) 359748. doi:10.1155/2014/359748.
- 605 [30] S.-M. Yie, H. Yang, S.-R. Ye, K. Li, D.-D. Dong, X.-M. Lin, Expression of HLA-G is
606 associated with prognosis in esophageal squamous cell carcinoma, *American Journal of*
607 *Clinical Pathology*. 128 (2007) 1002–1009. doi:10.1309/JNCW1QLDFB6AM9WE.
- 608 [31] M.-Y. Cai, Y.-F. Xu, S.-J. Qiu, M.-J. Ju, Q. Gao, Y.-W. Li, et al., Human leukocyte antigen-
609 G protein expression is an unfavorable prognostic predictor of hepatocellular carcinoma
610 following curative resection, *Clinical Cancer Research*. 15 (2009) 4686–4693.
611 doi:10.1158/1078-0432.CCR-09-0463.
- 612 [32] R.-L. Zhang, X. Zhang, S.-S. Dong, B. Hu, Q.-Y. Han, J.-G. Zhang, et al., Predictive value
613 of different proportion of lesion HLA-G expression in colorectal cancer, *Oncotarget*. 8
614 (2017) 107441–107451. doi:10.18632/oncotarget.22487.
- 615 [33] M.J. Rutten, F. Dijk, C.D. Savci-Heijink, M.R. Buist, G.G. Kenter, M.J. van de Vijver, et al.,
616 HLA-G expression is an independent predictor for improved survival in high grade ovarian
617 carcinomas, *Journal of Immunology Research*. 2014 (2014) 274584–11.
618 doi:10.1155/2014/274584.
- 619 [34] B. Davidson, M.B. Elstrand, M.T. McMaster, A. Berner, R.J. Kurman, B. Risberg, et al.,
620 HLA-G expression in effusions is a possible marker of tumor susceptibility to chemotherapy
621 in ovarian carcinoma, *Gynecol. Oncol*. 96 (2005) 42–47. doi:10.1016/j.ygyno.2004.09.049.
- 622 [35] L. König, S. Kasimir-Bauer, O. Hoffmann, A.-K. Bittner, B. Wagner, L.F.S. Manvailer, et al.,
623 The prognostic impact of soluble and vesicular HLA-G and its relationship to circulating
624 tumor cells in neoadjuvant treated breast cancer patients, *Hum. Immunol*. 77 (2016) 791–
625 799. doi:10.1016/j.humimm.2016.01.002.
- 626 [36] F. Morandi, R. Rizzo, E. Fainardi, N. Rouas-Freiss, V. Pistoia, Recent Advances in Our
627 Understanding of HLA-G Biology: Lessons from a Wide Spectrum of Human Diseases,
628 *Journal of Immunology Research*. 2016 (2016) 4326495–14. doi:10.1155/2016/4326495.

- 629 [37] F. Deschaseaux, J. Gaillard, A. Langonné, C. Chauveau, A. Naji, A. Bouacida, et al.,
630 Regulation and function of immunosuppressive molecule human leukocyte antigen G5 in
631 human bone tissue, *Faseb J.* 27 (2013) 2977–2987. doi:10.1096/fj.13-227264.
- 632 [38] W.N. Melsted, S.H. Matzen, M.H. Andersen, T.V.F. Hviid, The choriocarcinoma cell line
633 JEG-3 upregulates regulatory T cell phenotypes and modulates pro-inflammatory cytokines
634 through HLA-G, *Cellular Immunology.* 324 (2018) 14–23.
635 doi:10.1016/j.cellimm.2017.11.008.
- 636 [39] K.M. Elias, R.A. Harvey, K.T. Hasselblatt, M.J. Seckl, R.S. Berkowitz, Type I interferons
637 modulate methotrexate resistance in gestational trophoblastic neoplasia, *American Journal*
638 *of Reproductive Immunology.* 77 (2017) e12666–10. doi:10.1111/aji.12666.
- 639 [40] M.A. Olayioye, R.M. Neve, H.A. Lane, N.E. Hynes, The ErbB signaling network: receptor
640 heterodimerization in development and cancer, *Embo J.* 19 (2000) 3159–3167.
641 doi:10.1093/emboj/19.13.3159.
- 642 [41] D.J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, et al., Use of
643 chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that
644 overexpresses HER2, *The New England Journal of Medicine.* 344 (2001) 783–792.
645 doi:10.1056/NEJM200103153441101.
- 646 [42] G. Meinhardt, S. Kaltenberger, C. Fiala, M. Knöfler, J. Pollheimer, ERBB2 gene
647 amplification increases during the transition of proximal EGFR(+) to distal HLA-G(+) first
648 trimester cell column trophoblasts, *Placenta.* 36 (2015) 803–808.
649 doi:10.1016/j.placenta.2015.05.017.
- 650 [43] X. Yang, Z. Zhang, C. Jia, J. Li, L. Yin, S. Jiang, The relationship between expression of c-
651 ras, c-erbB-2, nm23, and p53 gene products and development of trophoblastic tumor and
652 their predictive significance for the malignant transformation of complete hydatidiform mole,
653 *Gynecol. Oncol.* 85 (2002) 438–444.
- 654 [44] B. Cameron, A.M. Gown, H.K. Tamimi, Expression of c-erb B-2 oncogene product in
655 persistent gestational trophoblastic disease, *The American Journal of Obstetrics &*
656 *Gynecology.* 170 (1994) 1616–21– discussion 1621–2.

- 657 [45] M. Hasanzadeh, N. Sharifi, H. Esmaili, M.S. Daloe, A. Tabari, Immunohistochemical
658 expression of the proliferative marker Ki67 in hydatidiform moles and its diagnostic value in
659 the progression to gestational trophoblastic neoplasia, *J. Obstet. Gynaecol. Res.* 39 (2012)
660 572–577. doi:10.1111/j.1447-0756.2012.01981.x.
- 661 [46] S. Yazaki-Sun, S. Daher, M.M. De Souza Ishigai, M.T.S. Alves, T.M. Mantovani, R. Mattar,
662 Correlation of c-erbB-2 oncogene and p53 tumor suppressor gene with malignant
663 transformation of hydatidiform mole, *J. Obstet. Gynaecol. Res.* 32 (2006) 265–272.
664 doi:10.1111/j.1447-0756.2006.00397.x.
- 665 [47] V. Fulop, S.C. Mok, D.R. Genest, I. Szigetvari, I. Cseh, R.S. Berkowitz, c-myc, c-erbB-2, c-
666 fms and bcl-2 oncoproteins. Expression in normal placenta, partial and complete mole, and
667 choriocarcinoma, *J Reprod Med.* 43 (1998) 101–110.

668 **Tables**

669 **Table 1.** Gestational choriocarcinoma-treated patients' characteristics

670 n, number; EMA-CO, etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine;
671 EMA-EP, etoposide, methotrexate, actinomycin D, etoposide and cisplatin; range, min-max; y,
672 year; hCG, human chorionic gonadotropin; IU/L, international units per liter; FIGO, Fédération
673 Internationale des Gynécologues et Obstétriciens;

674

675 FIGO stage I: disease is confined to the uterus, stage II: disease extends to the genital tract,
676 stage III: disease is spread to lungs with or without extension to the genital tract, and stage IV
677 occupies all other metastatic sites including liver, kidney, spleen, and brain.

678 *1 patient was cured by actinomycin D after first-line methotrexate failure.

679

680 **Table 2.** Choriocarcinoma samples used for transcriptomic profile comparisons

681 ^aincluding 2 patients with 2 biopsies on distinct metastatic sites

682 ^bincluding 1 patient with 2 biopsies on distinct metastatic sites

683

684 **Table 3.** Differentially expressed genes according to extremely (<0.1) and low
685 (<0.25) stringency false discovery rate (FDR) adjusted p-value thresholds.

686

687 A- 14 complete hydatidiform moles with subsequent malignant transformation in
688 choriocarcinoma were compared with 20 complete hydatidiform moles which
689 were followed by hCG normalization without chemotherapy.

690 B- 34 monochemoresistant were compared with 9 monochemosensitive
691 choriocarcinoma samples

692 C- 20 polychemoresistant were compared with 15 polychemosensitive
693 choriocarcinoma samples

694 **Figure caption**

695

696 **Figure 1. Localization and semi-quantitative assessment of HLA-G and ERBB2**

697 **protein expression in mono, or polychemotherapy resistant choriocarcinomas.**

698

699 **A. HLA-G immunostaining in gestational choriocarcinomas according to**
700 **the resistance profile to monochemotherapy.** Standard coloration
701 (hematoxylin, eosin and safran) shows triphasic gestational choriocarcinomas
702 (upper panels, magnification 10X). Expression pattern of HLA-G in
703 monochemotherapy resistant (lower left panel) and monochemotherapy
704 sensitive (lower right panel) choriocarcinomas.

705 **B. HLA-G protein semi-quantitative assessment.** The h-score was obtained
706 after multiplication of intensity score (0-4) and the percentage of stained cells
707 (0, 1 for 0-10%, 2 for 11-50% and 3 for $\geq 50\%$); IT, Intermediate trophoblast;
708 CT, cytotrophoblast; ST, syncytiotrophoblast.

709 **C. ERBB2 immunostaining in gestational choriocarcinomas according to**
710 **the resistance profile to polychemotherapy.** Standard coloration
711 (hematoxylin, eosin and safran) shows triphasic gestational choriocarcinomas
712 (upper panels, magnification 10X). Expression pattern of ERBB2 in
713 polychemotherapy resistant (lower left panel) and polychemotherapy sensitive
714 (lower right panel) choriocarcinomas.

715 **D. ERBB2 protein semi-quantitative assessment.** The h-score was obtained
716 after multiplication of intensity score (0-4) and the percentage of stained cells
717 (0, 1 for 0-10%, 2 for 11-50% and 3 for $\geq 50\%$); IT, Intermediate trophoblast;
718 CT, cytotrophoblast; ST, syncytiotrophoblast.

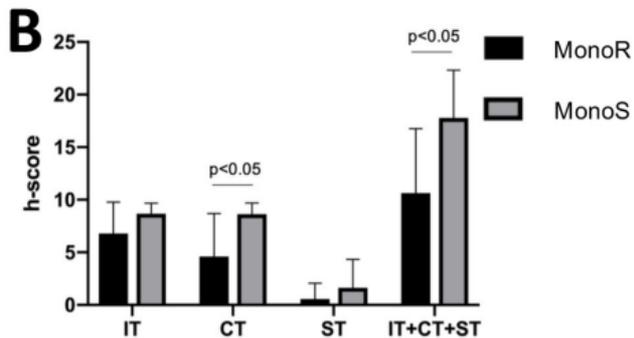
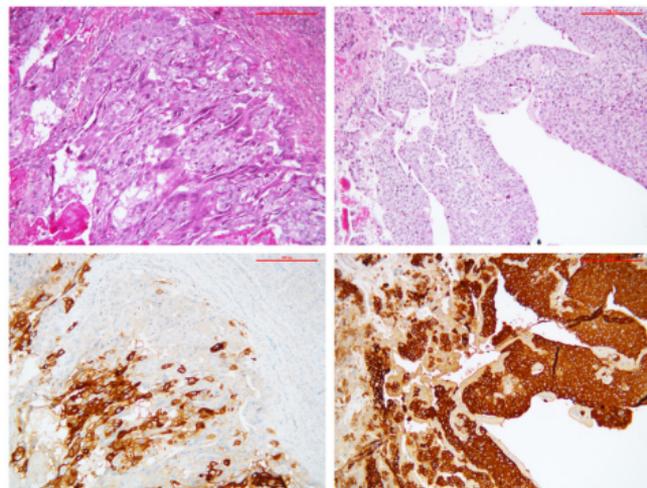
719 **Figure 2. Predicted affected biological functions and upstream regulators in**
720 **mono and polychemotherapy resistant gestational choriocarcinomas.**

721 The upper two panels (A) show the predicted inhibition (grey and blue boxes) of
722 down- (green boxes) and up- (red boxes) regulation of differentially expressed genes
723 on biological functions according to mono or polychemotherapy resistant profiles of
724 choriocarcinomas. The lower two panels (B) show the activation (orange square box)
725 or inhibition (blue square boxes) state of predicted upstream regulators of
726 differentially expressed genes according to mono or polychemotherapy resistant
727 profiles of choriocarcinomas.

728 Blue and orange arrows indicate relationships leading to inhibition and activation,
729 respectively, and yellow arrows indicate findings inconsistent with state of
730 downstream molecule. For grey arrows, the effect is not predicted.

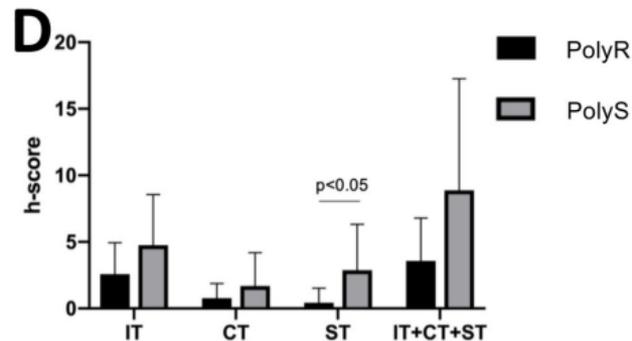
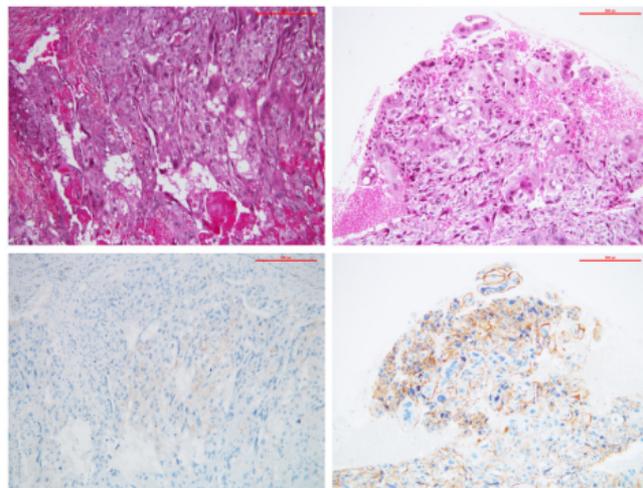
HLA-G

A Monochemotherapy resistant Monochemotherapy sensitive

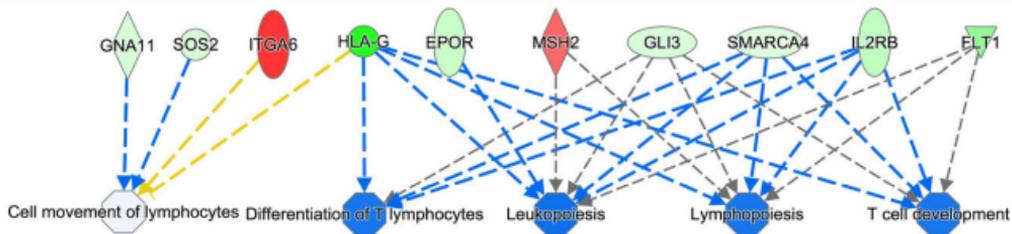


ERBB2

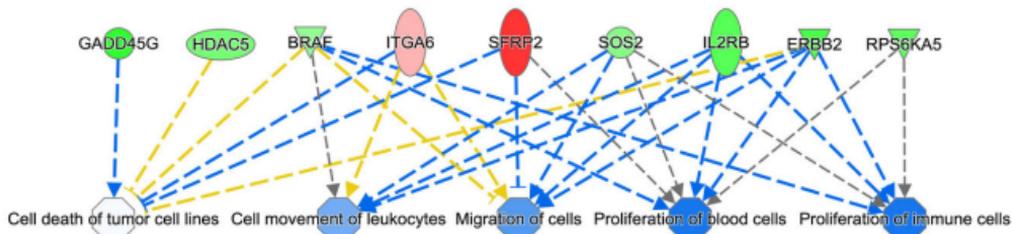
C Polychemotherapy resistant Polychemotherapy sensitive



A. Predicted affected biological functions

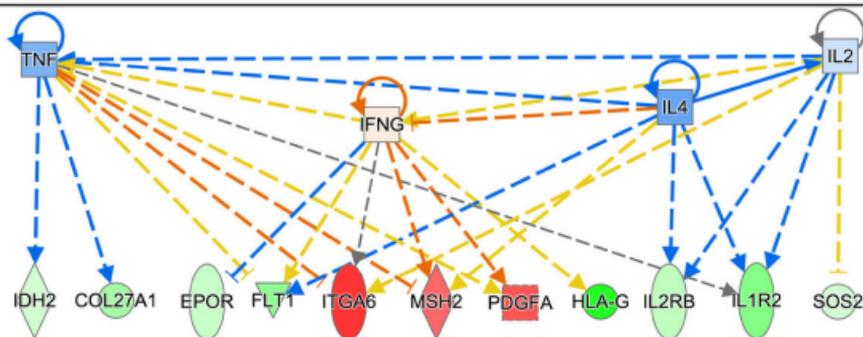


Monochemoresistant choriocarcinoma

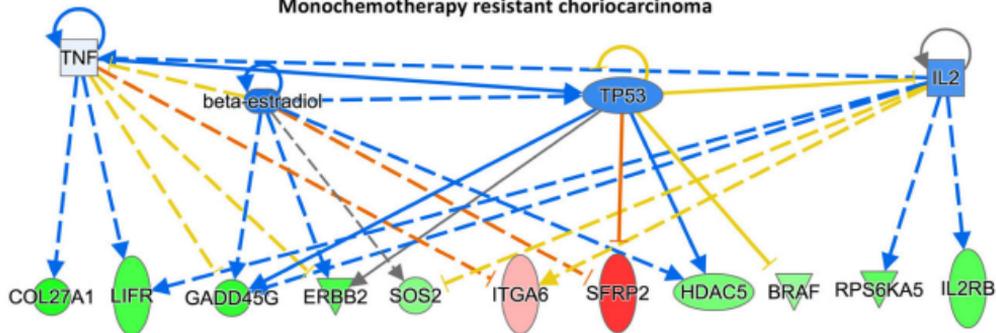


Polychemoresistant choriocarcinoma

B. Predicted upstream regulators



Monochemotherapy resistant choriocarcinoma



Polychemotherapy resistant choriocarcinoma

	first-line monochemotherapy		polychemotherapy (EMA-CO or EMA-EP)	
	monochemoresistant	monochemosensitive	polychemoresistant	polychemosensitive
n	14	9	18	14
mean age (range), y	36.4 (26.5-54.7)	37 (25.5-50.8)	33.7 (19.6-45.2)	35.2 (22.4-54.7)
median hCG (range), IU/L	28,875 (233-201,938)	14,422 (739-143,693)	61,670 (233-349,646)	109,135 (1,900-859,233)
initial FIGO score				
median (range)	5 (1-8)	3 (0-5)	8.5 (3-17)	8 (1-12)
0-4	6	8	2	3
5-6	7	1	4	3
7-12	1	0	6	8
≥13	0	0	6	0
initial FIGO stage				
I	12	6	7	9
II	0	2		
III	2	1	8	4
IV	0	0	3	1
index pregnancy				
delivery	5	0	10	5
abortion or ectopic pregnancy	3	0	5	3
hydatidiform mole	5	9	3	4
not determined	1	0	0	2
Interval from index pregnancy				
<4months	8	6	9	6
4-6 months	3	3	1	5
7-12 months	2	0	3	2
>12 months	1	0	5	1
median n of chemotherapy lines (range)	3 (2-6)	1 (1-2)*	3 (1-6)	1 (1-4)
median n of courses of monochemotherapy (range)	4 (3-8)	6 (3-9)	4.5 (4-8)	4 (4-5)
median n of courses of the first polychemotherapy (range)	5 (3-9)	0	6 (3-12)	6 (3-9)
death (%), n	1 (7)	0	10 (55)	0
Mean follow-up (range), month	49.7 (19.1-102.3)	17.06 (12.8-33.1)	30.9 (7.4-102.3)	34.2 (19.1-97.2)

Table 1. Gestational choriocarcinoma-treated patients' characteristics

n, number; EMA-CO, etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine; EMA-EP, etoposide, methotrexate, actinomycin D, etoposide and cisplatin; range, min-max; y, year; hCG, human chorionic gonadotropin; IU/L, international units per liter; FIGO, Fédération Internationale des Gynécologues et Obstétriciens;

FIGO stage I: disease is confined to the uterus, stage II: disease extends to the genital tract, stage III: disease is spread to lungs with or without extension to the genital tract, and stage IV occupies all other metastatic sites including liver, kidney, spleen, and brain.

*1 patient was cured by actinomycin D after first-line methotrexate failure.

	Resistant	Sensitive
Monochemotherapy (n)	34 choriocarcinoma	9 choriocarcinoma
	14 patients resistant to upfront monochemotherapy	9 patients sensitive to upfront monochemotherapy
	18 patients resistant to upfront polychemotherapy ^a	
Polychemotherapy (n)	20 choriocarcinoma	15 choriocarcinoma
	18 patients resistant to upfront polychemotherapy ^a	14 patients sensitive to upfront polychemotherapy ^b

Table 2. Choriocarcinoma samples used for transcriptomic profile comparisons

^aincluding 2 patients with 2 biopsies on distinct metastatic sites

^bincluding 1 patient with 2 biopsies on distinct metastatic sites

A. Post molar malignant transformation in choriocarcinoma

gene name	relative expression fold change	FDR adjusted p-value
CDKN1C	2,74	0,51
SOS1	1,15	0,52
CAMK2B	1,76	0,7
JUN	-3,24	0,7
KLF4	-1,54	0,8

B. Choriocarcinoma resistant to monochemotherapy

gene name	relative expression fold change	FDR adjusted p-value
HLA-G	-9.19	0.01
COL27A1	-3.65	0.02
IL1R2	-4.95	0.02
THEM4	1.55	0.02
GLI3	-1.59	0.05
PDGFA	1.75	0.05
FLT1	-4.53	0.08
IDH2	-1.8	0.08
SMARCA4	-1.26	0.08
CREB3L4	-1.33	0.09
GNA11	-1.58	0.09
IL2RB	-2.55	0.09
MSH2	1.56	0.09
EPOR	-2.22	0.11
ITGA6	2.12	0.15
SOS2	-1.72	0.15
HIST1H3G	-2.38	0.18
SUV39H2	-1.86	0.21
CACNA2D2	2.02	0.22

C. Choriocarcinoma resistant to polychemotherapy

gene name	relative expression fold change	FDR adjusted p-value
SOS2	-1,61	0,07
ERBB2	-2,44	0,08
ITGA6	1,79	0,08
LIFR	-2,47	0,08
GADD45G	-2,75	0,09
SFRP2	4,87	0,12
BRAF	-1,43	0,15
IL2RB	-2,26	0,15
RPS6KA5	-2,16	0,15
COL27A1	-3	0,17
HDAC5	-1,83	0,18
TCL1B	-2,93	0,18

Table 3. Differentially expressed genes according to extremely (<0.1) and low (<0.25) stringency false discovery rate (FDR) adjusted p-value thresholds.

- A- 14 complete hydatidiform moles with subsequent malignant transformation in choriocarcinoma were compared with 20 complete hydatidiform moles which were followed by hCG normalization without chemotherapy.
- B- 34 monochemoresistant were compared with 9 monochemosensitive choriocarcinoma samples
- C- 20 polychemoresistant were compared with 15 polychemosensitive choriocarcinoma samples