Chemotherapy-induced changes in bronchoalveolar lavage fluid CD4+ and CD8+ cells of the opposite lung to the cancer

Georgia Karpathiou1, Vasilios Mihailidis1, Evangelia Nakou2, Stavros Anevlavis1, Argyrios Tzouvelekis2, George Kouliatsis2, Paschalis Ntolios1, Demosthenes Bouros1, Ioannis Kotsianidis2 & Marios E. Froudarakis1

Published articles support the effect of chemotherapy in the immune environment of tumors, including lung carcinomas. The role of CD4+ T-cells is crucial for expansion and accumulation of other antigen-specific immune cells, and the participation of CD8+ cells in tumor killing activity has been confirmed by many studies. However, little is known about the effect of chemotherapy on the healthy lung parenchyma from lung cancer patients, and whether there are differences between the different chemotherapy compounds used to treat this patient population. The aim of our study was to explore the effect of chemotherapy on CD4+ and CD8+ cells in the bronchoalveolar lavage fluid (BALF) of the healthy lung in patients treated with standard chemotherapy regimens. Fifteen patients underwent BAL, in the healthy lung before and after six chemotherapy courses. Platinum-based regimens included vinolerbine (VN) in 6 patients, gemcitabine (GEM) in 4 patients and etoposide (EP) in 5 patients. All patients but one were males and smokers (93%). The median age of patients was 56 years (42–75). No significant difference was noted in the patients’ age between the three treated groups. Furthermore, between the three groups, no significant changes in the means of CD4+ and CD8+ cells were noted. However, when we compared the mean CD4+ cells before and after chemotherapy within each group, changes were noted when comparing VN before versus after (p = 0.05), GEM before versus after (p = 0.03), and EP before versus after (p = 0.036). In our pilot study, changes were noted in BALF CD4+ cells for the three most applied regimens at the normal lung parenchyma.

Changes in the local immune microenvironment play an important role in cancer development. Studies have shown a positive prognostic role of tumor-infiltrating cytotoxic (CD8+) T lymphocytes in various forms of cancer including lung tumors1–3. This ability of the immune system to act against tumor cells is the basis of immunotherapy and the immune system status has a predictive role for this type of therapy. Furthermore, immune system components can be affected by cancer treatments like chemotherapy or radiotherapy, which can act as immunosuppressants reducing lymphocytic counts, or as factors that through necrosis of tumor cells, induce the release of tumor antigens and facilitate the recruitment of immune cells4.

The components of the immune system can be evaluated in three different compartments, the peripheral blood, the organs and the tumor itself inside an organ. One well-established method of lung microenvironment investigation is bronchoalveolar lavage (BAL). BAL is a safe non-invasive method that permits one to analyze the local immune response in a large number of lung diseases5,6. Studies have shown that lung tumors result in high CD4+ T lymphocyte counts in BAL fluid (BALF) from the affected lung. In addition, after radiation therapy for mammary carcinoma, the increase of lymphocytes found in patients’ BALF mainly consisted of activated cluster of differentiated CD4+ cells migrating from the irradiated lung to the contralateral one7,8. Chemotherapy is another cause of lung damage in cancer patients9. We have previously shown that early changes of the lung parenchyma may occur without radiology findings in transbronchial biopsies from lung cancer patients treated with chemotherapy10. Yet, the effects of chemotherapy in the local immune microenvironment of the opposite

1Department of Respiratory Medicine, Medical School of Alexandroupolis, Democritus University of Thrace, Alexandroupolis, Greece. 2Department of Hematology, Medical School of Alexandroupolis, Democritus University of Thrace, Alexandroupolis, Greece. *email: mfroud@med.duth.gr
lungs (to the affected lung) have not been studied in patients with lung cancer, especially with regards to compounds used in a prospective way.

Thus, the aim of this study is to explore possible changes of CD4+ and CD8+ lymphocytes in the BALF of the opposite (healthy) lung after administration of three classic platinum combinations with gemcitabine, etoposide and vinorelbine, used for the treatment of lung cancer patients, in order to assess if these agents can affect the immune microenvironment of the healthy lung.

Materials and methods

This is a prospective study conducted at the University Hospital, Medical School Democritus University of Thrace after the approval was obtained from the internal Review Board (IRBN 4360/01/11/2007), according to the Greek regulations for good clinical practice. It is part of a research protocol on lung toxicity from chemotherapy and radiation therapy from which two articles have already been published. All patients signed informed consent prior to the study. Inclusion criteria were: patients histologically diagnosed with lung cancer, stage IIIB or IV, Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1, adequate imaging, and no severe comorbidity. Any progression or PS deterioration or serious adverse event including grade III/IV toxicity resulted in the patient's withdrawal from the study. Finally, 15 patients were included and concluded in the study. They were treated with chemotherapy, prior to radiotherapy, of one of the three platinum-based chemotherapy groups: cisplatin–vinorelbine (VN) (6 patients), cisplatin-gemcitabine (GEM) (4 patients) or cisplatin-etoposide (EP) (5 patients). The first group received 30 mg/m² of VN, administered on days 1 and 8. The second group received 1000 mg/m² of GEM on days 1 and 8 and the third group received 150 mg/m² of EP on days 1–3. All three compounds were given with cisplatin on day 1 at a dose of 100 mg/m². Treatment was repeated in each group every 3 weeks for a total of six planned courses.

BAL was conducted in the lung opposite the tumor lung before treatment (T0) during diagnostic bronchoscopy and at the end of six cycles of chemotherapy (T1). During the procedure, 100 ml of 0.9% NaCl solution was instilled at the upper lobe of the contralateral to the tumor lung. The recovery fluid was filtered and centrifuged for 10 min (300 g). For flow cytometry, fresh mononuclear BAL cells were stained with the following antibodies after red cell lysis with ammonium chloride: CD3 (HIT3a), CD4 (RPA-T4), CD8 (RPA-T8), CD45(2D1), and the appropriate isotypic controls, all from BD Pharmingen. Data acquisition and analysis was performed on a FACSCalibur, equipped with CellQuest Pro software (BD biosciences). Treg frequency is reported as percentage of CD4+ cells. The cell pellet was used for counting and for flow cytometry with determination of T lymphocyte immunophenotypes after staining with surface anti-CD3+, anti-CD45, figoanti-CD4+, and CD8+ antibodies (Fig. 1), as previously described.

Statistical analysis was performed with a statistical software package (StatView version 4.5, Abacus Concepts Inc., Berkeley, CA, USA). CD4+ and CD8+ counts were expressed as mean values ± standard deviation (±SD). Comparison of means between groups was performed with the Student’s t-test. The analysis of variances (ANOVA) test was used to determine either whether a compound was a factor of difference (factorial analysis) or
the values between the different time points within the same group or different groups (repeated measurements analysis). Statistical significance of all tests was set at a $p$ value of less than 0.05.

**Results**

Fourteen patients were male (93.3%) and one was female (6.7%). Mean age was 58 years ($\pm 9.4$). All patients but one were smokers (93.3%). Ten patients were diagnosed with non-small-cell lung cancer (NSCLC) and five with small-cell lung cancer (SCLC). Patients demographics according to treatment schedule are shown in Table 1.

CD4+ and CD8+ counts did not differ significantly between the three chemotherapy groups neither before nor after chemotherapy (Fig. 2). The CD4+ count showed changes after chemotherapy (Table 3) for vinorelbine ($p = 0.05$), gemcitabine ($p = 0.03$), and etoposide ($p = 0.036$), as CD4+ positive lymphocytes increased after VB chemotherapy, while they decreased after GM and EP chemotherapy (Fig. 2). No significant changes were found for CD8+ counts.

When the difference (delta) of the value was calculated between the value of the lymphocyte subsets before chemotherapy and the value after chemotherapy and the means were compared between the different groups (EP vs. VN vs. GEM), there was a significant difference between only CD4+ of the VN group and CD4+ of the EP group ($p = 0.002$, Fig. 3).

**Discussion**

The literature supports the effect of chemotherapy in the immune environment of the tumors, including lung carcinomas. However, little is known on the effect of chemotherapy on the lung parenchyma opposite the cancer lung, and whether there are specific differences between the different chemotherapy compounds used to treat this patient population. The hypothesis of our study was whether there are differences in the way that various agents act on the lung immune microenvironment that may explain the behavior of the tumor itself regarding these agents. We found that after treatment of three usual platinum combinations in patients with lung cancer, there are differences between gemcitabine (GEM), etoposide (EP) and vinorelbine (VN). It seems that, when added to platinum, VN increases the counts of CD4+ subsets while EP and GEM have the opposite action. In addition, CD8+ subpopulation was decreased in VN and GEM combinations, although it was not statistically significantly, while it remained unchanged in the EP one.

The role of CD4+ helper T-cells is crucial for efficient expansion and accumulation of other antigen-specific immune cells. CD4+ Th1 cells secrete type I cytokines, such as IFN-γ and TNF-α, which stimulate CD8+ T cell responses via activation of antigen-presenting cells. CD4+ Th2 cells secrete type II cytokines, such as IL-4, IL-5 and IL-13, which limit the activation of antigen-presenting cells, but may enhance humoral immunity. It is generally considered that Th1high/Th2low is a good indicator of an anti-tumor response. In NSCLC, Huang and collaborators reported that Th1 subsets significantly decrease in tumors, suggesting that the Th1 type immune response is decreased.

---

**Table 1.** Patients characteristics according to the treatment schedule.

|                | Total         | Vinorelbine | Gemcitabine | Etoposide | $p$  |
|----------------|---------------|-------------|-------------|-----------|-----|
| Age            | 58 ± 9.4      | 61.1 ± 7.8  | 55 ± 11     | 56.6 ± 10.9 | 0.6 |
| Pack-years     | 76.4 ± 29.4   | 82.0 ± 20.2 | 57.5 ± 46.4 | 85.0 ± 20  | 0.34|
| NSCLC          | 10 (66.6%)    | 6           | 4           | 0         | 0.83|
| ADK            | 4             | 1           | 3           |           |     |
| SqC            | 4             | 3           | 1           |           |     |
| LCC            | 2             | 2           | 0           |           |     |
| SCC            | 5 (23.3%)     |             |             | 5         | 0.09|
| Stage NSCLC    |               |             |             |           |     |
| III            | 7 (46.6%)     | 3           | 4           |           |     |
| IV             | 3 (20%)       | 3           | 0           |           |     |
| Stage SCLC     |               |             |             |           |     |
| Limited        | 4 (26.6%)     |             |             | 5         |     |
| Extensive      | 1 (6.6%)      |             |             |           |     |

**Table 2.** Comparison between the three groups of CD4+ and CD8+ counts (mean ± standard deviation) before (T0) and after (T1) chemotherapy.

|                | Vinorelbine | Etoposide | Gemcitabine | $p$  |
|----------------|-------------|-----------|-------------|-----|
| CD4+ (T0)     | 27.5 ± 22.5 | 42.6 ± 9.2 | 57 ± 5.6   | 0.2 |
| CD8+ (T0)     | 41.1 ± 19.7 | 37.1 ± 11 | 29.5 ± 0.7 | 0.68|
| CD4+ (T1)     | 47.3 ± 30   | 27.5 ± 19.7 | 45.2 ± 13 | 0.41|
| CD8+ (T1)     | 30 ± 19     | 37.6 ± 15 | 16.3 ± 2   | 0.24|
response is weakened in tumor sites\(^1\). In our study, although we found that the total number of CD4 + cells did not change after chemotherapy, when we analyzed our subgroups of regimens, the CD4 + cell count in the VN-platinum association increased significantly after chemotherapy, while the CD4 + cell count in GEM and EP combinations decreased. Other investigators reported fewer T-helper cells in tumor infiltrating lymphocytes\(^1\) or in BALF of patients with lung cancer compared to controls\(^6\). However, these studies are performed in surgically treated patients with NSCLC, which directly assess the local tumor microenvironment by immunohistochemistry, in terms of prognostic significance\(^3\), or the BALF of the affected lung\(^6,17\). In addition, the affected pulmonary parenchyma may also suffer from local infection, due to tumor obstruction, thus, affecting local immunity.

The role of \(T\) cytotoxic cells in lung cancer has been well documented. The participation of CD8 + cells in tumor killing activity has been confirmed since the mid-90s by Fujisawa et al.\(^19\) and Burger et al.\(^20\). Yoshino and associates\(^15\) reported an increased proportion of CD8 + cells in tumor infiltrating lymphocytes and Domagala-Kulawik et al.\(^16\) in BALF, whereas a decrease was observed in the peripheral blood\(^16\). In our study, the proportion of these cells in the BALF from patients with lung cancer was slightly decreased after chemotherapy. When we looked at our subgroups, this decrease concerned mainly the VN and GEM combinations, while the EP combination remained unchanged. It is also important to consider the influence of cigarette smoking on CD8 + count,

![Figure 2](link)

**Table 3.** Comparison of CD4 + and CD8 + counts (mean ± standard deviation) between before (T0) and after (T1) chemotherapy according to the chemotherapy regimen.

| Regimen  | \(T0\)         | \(T1\)         | \(p\)  |
|----------|----------------|----------------|-------|
| **Total**|                |                |       |
| CD4 +    | 39.1 ± 19      | 39.3 ± 21.3    | 0.57  |
| CD8 +    | 37.1 ± 14.1    | 28 ± 15.5      | 0.59  |
| **Vinorelbine** |        |                |       |
| CD4 +    | 27.5 ± 22.5    | 47.3 ± 30      | 0.05  |
| CD8 +    | 41.1 ± 19.7    | 30 ± 1         | 0.8   |
| **Gemcitabine** |        |                |       |
| CD4 +    | 57 ± 5.6       | 45.2 ± 13      | 0.03  |
| CD8 +    | 29.5 ± 0.7     | 16.3 ± 2       | 0.11  |
| **Etoposide** |        |                |       |
| CD4 +    | 42.6 ± 9.2     | 27.5 ± 19.7    | 0.036 |
| CD8 +    | 37 ± 11        | 37.6 ± 15      | 0.56  |
which may bias these data. Indeed, Costabel and collaborators in the 1980s\(^5\) showed an increase in the proportion of these cells in the BALF of smokers.

Lung parenchyma toxicity after chemotherapy also has to be considered when trying to define the tumor immune microenvironment. Bhalla et al.\(^21\) studied BALF in nine patients with breast cancer receiving four cycles of induction chemotherapy and five healthy controls, showing higher percentages of neutrophils and lymphocytes in the first group. Their study showed that the patients suffered from pulmonary toxicity, according to their lung function tests and the lung inflammatory infiltration, studied by BAL\(^21\). Similarly, we have previously shown that transbronchial biopsies from the healthy lung parenchyma in patients with lung cancer showed early histological changes after chemotherapy and radiation therapy\(^10\). This was associated with changes in respiratory function tests\(^11\). These studies show that the healthy lung parenchyma is influenced by chemoradiotherapy and should be studied separately from the cancer-affected lung.

Parra and associates\(^22\) studied the tumor tissue microenvironment in patients with NSCLC who underwent neoadjuvant chemotherapy versus no chemotherapy, with further surgical resection. They showed higher densities of CD4\(^+\) cells in the pretreated group\(^22\). Cytotoxic T cells (CD8\(^+\)) were assessed by Pasello and investigators\(^23\) in malignant pleural mesotheliomas (MPM) prior to chemotherapy. Patients who achieved response or stable disease showed lower CD8\(^+\) cells compared to those with progressive disease\(^23\). They reported that higher levels of cytotoxic T lymphocytes remaining after chemotherapy in MPM suggested a more aggressive tumor with lower chances of response to chemotherapy\(^23\). In rectal cancer, Matsutani et al.\(^4\) reported that CD8\(^+\) lymphocytes in tissue specimens increased after neoadjuvant chemoradiotherapy but not neoadjuvant chemotherapy.

In breast cancer, where neoadjuvant therapy is often the cornerstone of treatment, a decrease of CD4\(^+\) T lymphocytes\(^24\), and an increase of CD8\(^+\) lymphocytes\(^24,25\) in comparison to the pre-treatment tissue specimen has been reported, suggesting an increase in tumor antigenicity after neoadjuvant chemotherapy\(^24,25\). Ladoire et al.\(^26\), on the contrary, did not observe any change in CD8\(^+\) infiltrates using a semi-quantitative scoring. Also in breast cancer patients, a 15% decrease in tumor-infiltrating lymphocytes after chemotherapy was reported by Pelekanou et al.\(^27\).

The reasons for these discrepancies are unclear, but a contribution of chemotherapy regimen cannot be ruled out, since immune effects differ between different drugs\(^28\), as was the case in our study. These observations highlight the difficulties in studying the immune microenvironment after treatment because tumor-associated characteristics, treatment differences, or even temporal changes related to treatment schedule, may influence the immune system. In our study, as we are interested in the behavior of the lung opposite the cancer lung, we minimized any tumor-related effect, as by definition this side was not influenced by the tumor. We also minimized this by setting the same time point of investigation at the end of chemotherapy.

Our study has limitations such as the small number of patients enrolled, the lack of subtyping the CD4\(^+\) cells, and the absence of follow-up data to compare with overall survival (OS) and progression-free survival (PFS).
Indeed, it is difficult to enroll patients with advanced stage lung carcinoma in such a study, even if bronchoscopy is the standard of care for diagnosis and staging the disease, because they will undergo many other investigations and therapeutic interventions during their disease management. In addition, the study was designed to report the differences in T-cell subpopulations and not their prognostic significance with the concern to assess only the microenvironment of the healthy lung parenchyma according to the chemotherapeutic regimen utilized to treat patients with advanced stage lung carcinoma.

To conclude, our pilot study of BAL in the lung parenchyma opposite the cancer lung, showed changes of CD4+ T-lymphocytes, while CD8+ T-lymphocytes showed no significant changes. These results should generate further studies comparing the effect of chemotherapy in healthy lung parenchyma to the effect on tumor microenvironment.

Data availability
Data are available upon request.

Received: 28 March 2020; Accepted: 26 October 2020
Published online: 16 November 2020

References
1. Karpatioiu, G. et al. Clinical and histologic predictive factors of response to induction chemotherapy in head and neck squamous cell carcinoma. *Am. J. Clin. Pathol.* **146**(3), 546–553 (2016).
2. Karpatioiu, G. et al. Prognostic impact of immune microenvironment in laryngeal and pharyngeal squamous cell carcinoma: immune cell subtypes, immuno-suppressive pathways and clinicopathologic characteristics. *Oncotarget* **8**(12), 19310–19322 (2017).
3. Kadota, K. et al. Prognostic impact of immune microenvironment in lung squamous cell carcinoma: tumor-infiltrating CD10+ neutrophil/CD20+ lymphocyte ratio as an independent prognostic factor. *J. Thorac. Oncol.* **10**(9), 1301–1310 (2015).
4. Matsutani, S. et al. Significance of tumor-infiltrating lymphocytes before and after neoadjuvant therapy for rectal cancer. *Cancer Sci.* **109**(4), 966–979 (2018).
5. Costabel, U., Bross, K. J., Reuter, C., Ruhle, K. H. & Matthys, H. Alterations in immunoregulatory T-cell subsets in cigarette smokers. A phenotypic analysis of bronchoalveolar and blood lymphocytes. *Chest* **90**(1), 39–44 (1986).
6. Osińska, I., Štemmaszczyk-Emmel, A., Polubicz-Kownacka, M., Dziedzic, D. & Domagala-Kulawik, J. CD4+/CD25(high)/FoxP3+/CD127- regulatory T cells in bronchoalveolar lavage fluid of lung cancer patients. *Hum. Immunol.* **77**(10), 912–915 (2016).
7. Roberts, C. M. et al. Radiation pneumonitis: a possible lymphocyte-mediated hypersensitivity reaction. *Ann. Intern. Med.* **118**(9), 696–700 (1993).
8. Martin, C. et al. Bilateral lymphocytic alveolitis: a common reaction after unilateral thoracic irradiation. *Eur. Respir. J.* **13**(4), 727–732 (1999).
9. Froudaρakis, M. et al. Revisiting bleomycin from pathophysiology to safe clinical use. *Crit. Rev. Oncol. Hematol.* **87**(1), 90–100 (2013).
10. Karpatioiu, G. et al. Histological changes after radiation therapy in patients with lung cancer: a prospective study. *Anticancer Res.* **34**(6), 3119–3124 (2014).
11. Mihailescu, V. et al. Lung function changes after chemoradiation therapy in patients with lung cancer treated by three usual platinum combinations. *J. Thorac. Dis.* **10**(9), 5435–5442 (2018).
12. Kostianidou, I. et al. Global impairment of CD4+CD25+FOXP3+ regulatory T cells in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* **179**(12), 1121–1130 (2009).
13. Gajewski, T. F., Schreiber, H. & Fu, Y. X. Innate and adaptive immune cells in the tumor microenvironment. *Nat. Immunol.* **14**(10), 1014–1022 (2013).
14. Huang, J., Shen, F., Huang, H., Ling, C. & Zhang, G. T.thigh in tumor microenvironment is an indicator of poor prognosis for patients with NSCLC. *Oncotarget* **8**(6), 13116–13125 (2017).
15. Yoshino, I. et al. Phenotypes of lymphocytes infiltrating non-small cell lung cancer tissues and its variation with histological types of cancer. *Lung Cancer* **10**(1–2), 13–19 (1993).
16. Domagala-Kulawik, J. et al. T-cell subtypes in bronchoalveolar lavage fluid and in peripheral blood from patients with primary lung cancer. *Diagn. Cytopathol.* **25**(4), 208–213 (2001).
17. Kwiecien, I., Stelmaszczyk-Emmel, A., Polubicz-Kownacka, M., Dziedzic, D. & Domagala-Kulawik, J. Elevated regulatory T cells, surface and intracellular CTLA-4 expression and interleukin-17 in the lung cancer microenvironment in humans. *Cancer Immunol. Immunother.* **66**(2), 161–170 (2017).
18. Domagala-Kulawik, J., Guzman, J. & Costabel, U. Immune cells in bronchoalveolar lavage in peripheral lung cancer—analysis of 140 cases. *Respiration* **70**(1), 43–48 (2003).
19. Fujisawa, T. & Yamaguchi, Y. Autologous tumor killing activity as a prognostic factor in primary resected non-small cell carcinoma of the lung. *Cancer* **79**(3), 474–481 (1997).
20. Burger, U. L., Chang, M. P., Goedegebuure, P. S., Eberlein, T. J. & Adams-Hodgins, S. Recruitment of host CD8+ T cells by tumor-infiltrating lymphocytes and recombinant interleukin-2 during adoptive immunotherapy of cancer. *Surgery* **117**(3), 325–333 (1995).
21. Bhalla, K. S. et al. Pulmonary toxicity of induction chemotherapy prior to standard or high-dose chemotherapy with autologous hematopoietic support. *Am. J. Respir. Crit. Care Med.* **161**(1), 17–25 (2000).
22. Parra, E. R. et al. Effect of neoadjuvant chemotherapy on the immune microenvironment in non-small cell lung carcinomas as determined by multiplex immunofluorescence and image analysis approaches. *J. Immunother. Cancer* **6**(1), 48 (2018).
23. Pasello, G. et al. Malignant pleural mesothelioma immune microenvironment and checkpoint expression: correlation with clinical-pathological features and intratumor heterogeneity over time. *Ann. Oncol.* **29**(5), 1258–1265 (2018).
24. Garcia-Martinez, E. et al. Tumor-infiltrating immune cell profiles and their change after neoadjuvant chemotherapy predict response and prognosis of breast cancer. *Breast Cancer Res* **18**(6), 488 (2014).
25. Goto, W. et al. Predictive value of improvement in the immune microenvironment in patients with breast cancer treated with neoadjuvant chemotherapy. *ESMO Open* **3**(6), e000305 (2018).
26. Ladoire, S. et al. Pathologic complete response to neoadjuvant chemotherapy of breast carcinoma is associated with the disappearance of tumor-infiltrating foxp3+ regulatory T cells. *Clin. Cancer Res.* **14**(8), 2413–2420 (2008).
27. Polekanou, V. et al. Tumor-infiltrating lymphocytes and PD-1/L1 expression in pre- and posttreatment breast cancers in the SWOG S0800 phase II neoadjuvant chemotherapy trial. *Mol. Cancer Ther.* **17**(6), 1324–1331 (2018).
28. Diis, M. L. & Stanton, S. E. Can immunity to breast cancer eliminate residual micrometastases?. *Clin. Cancer Res.* **19**(23), 6398–6403 (2013).
Author contributions
Author contributions Section (I) M.E.F., G.K., V.M.: Conception and design. (II) All authors: Administrative support (III) All authors: Provision of study materials or patients (IV) All authors: Collection and assembly of data (V) All authors: Data analysis and interpretation (VI) All authors: Manuscript writing (VII) All authors: Final approval of manuscript.

Funding
This research was supported with a grant from “Fondation Lancardis”, Martigny, Switzerland CH-1920.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to M.E.F.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020