

# Prevention of radiotherapy-induced arterial inflammation by interleukin-1 blockade

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## Aims

Radiotherapy-induced cardiovascular disease is an emerging problem in a growing population of cancer survivors where traditional treatments, such as anti-platelet and lipid-lowering drugs, have limited benefits. The aim of the study was to investigate vascular inflammatory patterns in human cancer survivors, replicate the findings in an animal model, and evaluate whether interleukin-1 (IL-1) inhibition could be a potential treatment.

## Methods and results

Irradiated human arterial biopsies were collected during microvascular autologous free tissue transfer for cancer reconstruction and compared with non-irradiated arteries from the same patient. A mouse model was used to study the effects of the IL-1 receptor antagonist, anakinra, on localized radiation-induced vascular inflammation. We observed significant induction of genes associated with inflammasome biology in whole transcriptome analysis of irradiated arteries, a finding supported by elevated protein levels in irradiated arteries of both, pro-caspase and caspase-1. mRNA levels of inflammasome associated chemokines *CCL2*, *CCL5* together with the adhesion molecule *VCAM1*, were elevated in human irradiated arteries as was the number of infiltrating macrophages. A similar pattern was reproduced in *Apoe*<sup>-/-</sup> mouse 10 weeks after localized chest irradiation with 14 Gy. Treatment with anakinra in irradiated mice significantly reduced *Ccl2* and *Ccl5* mRNA levels and expression of I-A<sup>b</sup>.

## Conclusion

Anakinra, administered directly after radiation exposure for 2 weeks, ameliorated radiation induced sustained expression of inflammatory mediators in mice. Further studies are needed to evaluate IL-1 blockade as a treatment of radiotherapy-induced vascular disease in a clinical setting.

## Keywords

Anakinra • Interleukin-1 • MCP-1 • CCL2 • Vascular disease • Radiation

## Introduction

Several cancer therapies have recently been identified as risk factors for CVD in the growing population of cancer survivors.<sup>1–3</sup> Radiotherapy, a cornerstone in cancer treatment, inevitably involves exposure of healthy surrounding tissues.<sup>1</sup> Accumulating clinical

evidence demonstrate an increased risk for cardiovascular mortality and morbidity years after exposure to radiation, e.g. myocardial infarction after breast cancer and Hodgkin's lymphoma, and stroke after head and neck cancer.<sup>4–6</sup> In contrast, radiotherapy has been used prophylactically against restenosis after coronary angioplasty, but its usefulness is limited by late adverse effects that include

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## Translational perspectives

Irradiated human conduit arteries show sustained inflammation years after radiotherapy exposure, which can explain development of radiotherapy-induced cardiovascular disease (CVD) long time after exposure in cancer survivors. The current study shows elevated levels of inflammation signals associated with inflammasome activation in irradiated human blood vessels long after radiotherapy and replicates results from irradiated human conduit arteries in a mouse model of localized irradiation to the heart and carotids. In the model, the localized inflammatory response was ameliorated by treatment with anakinra, an interleukin-1 receptor antagonist in clinical use for treatment of rheumatoid arthritis. The findings here, combining analysis of irradiated human blood vessels and an interventional murine model, can further increase our understanding of radiotherapy-induced CVD in the emerging field on oncocardiology.

restenosis and vascular occlusion.<sup>7</sup> Animal experiments show that traditional drugs used for CVD, such as anti-platelet drugs and statins, have limited preventive effects on radiation-induced CVD.<sup>2,3</sup> Therefore, it is urgent to identify new treatment modalities.

The mechanisms involved in human radiotherapy-induced CVD are poorly understood, largely because adequate samples for study are very difficult to obtain. We previously showed that irradiation of human arteries induces a sustained inflammation and chronic activation of nuclear factor kappa B (NF- $\kappa$ B)<sup>8</sup> which promotes transcription of pro-inflammatory cytokines including interleukin (IL)-1 $\alpha$  and IL-1 $\beta$ . Interleukin-1 $\alpha$  is both an inducer and a product of NF- $\kappa$ B activation<sup>9</sup> and IL-1 $\beta$  is up-regulated in irradiated human arteries.<sup>10</sup> The IL-1 receptor antagonist (IL-1Ra) is an endogenous inhibitor of IL-1 signalling and recombinant IL-1Ra (anakinra) is approved for treatment of rheumatoid arthritis.<sup>11</sup> Endogenous IL-1Ra is cardioprotective by reducing apoptosis and myocardial infarction size in mice<sup>12</sup> and anti-IL-1 therapy is used in clinical trials of CVD treatment.<sup>11,13,14</sup> However, anti-IL-1 therapy has never been studied in radiation-induced vascular disease.

We hypothesized that reduction of the inflammatory response early after radiation reduces the sustained chronic inflammation associated with late adverse effects. To investigate this, we studied the inflammation in human chronic radiation injury. We used a mouse model of vascular irradiation damage that shares the sustained up-regulation of inflammatory mediators observed in irradiated human arteries and studied anti-IL-1 as a potential treatment for radiation-induced arterial inflammation in mice.

In radiation-induced chronic human arterial inflammation, we found increased levels of caspase-1 and induction of inflammasome-related genes. Treatment of irradiated mice with anti-IL-1 therapy reduced signs of inflammation in irradiated arteries. Hence, anti-IL-1 treatment may be a therapeutic approach for radiotherapy-induced vascular disease.

## Methods

### Human

Samples were obtained from the Biobank of Radiated tissues at Karolinska (BiRKA), including irradiated and non-irradiated paired arterial biopsies (Supplementary material online, Tables S1 and S2), harvested from the same patient at the same occasion during microvascular autologous free tissue transfers for cancer reconstructions, but with a shift towards more chronically radiation injured subjects than previously presented.<sup>14</sup>

### Animal radiation and treatment

Nine to ten weeks old atherosclerosis-prone apolipoprotein E deficient (*Apoe*-/-) females on a C57Bl/6N background ( $n = 39$ , Taconic,

Denmark) were fed with a standard chow diet. After exposure to X-rays or sham radiation, mice were injected intraperitoneally daily with either 100 mg/kg anakinra (Kineret<sup>®</sup>, Swedish Orphan Biovitrum, Stockholm, Sweden) diluted in 0.9% sodium chloride or equivalent volume sodium chloride only to controls for 2 weeks.

### Study approval

The study was approved by the Ethical Committee of Stockholm and was performed in agreement with institutional guidelines and the principles of the Declaration of Helsinki. All enrolled subjects gave informed consent. All animal experiments were approved by Stockholm Regional Board for Animal Ethics.

An expanded methods section is available in the [Supplementary material online](#).

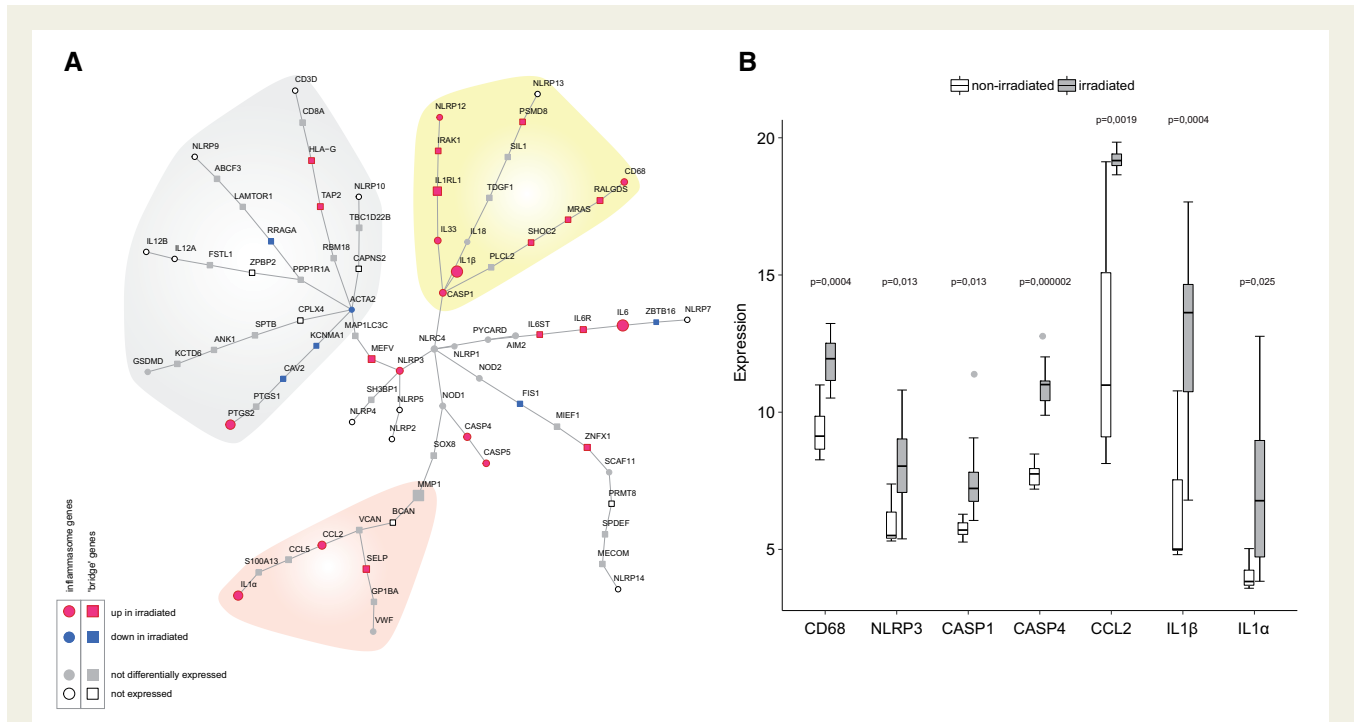
## Results

### Elevated levels of inflammasome-related transcripts in human arteries with chronic radiation injury

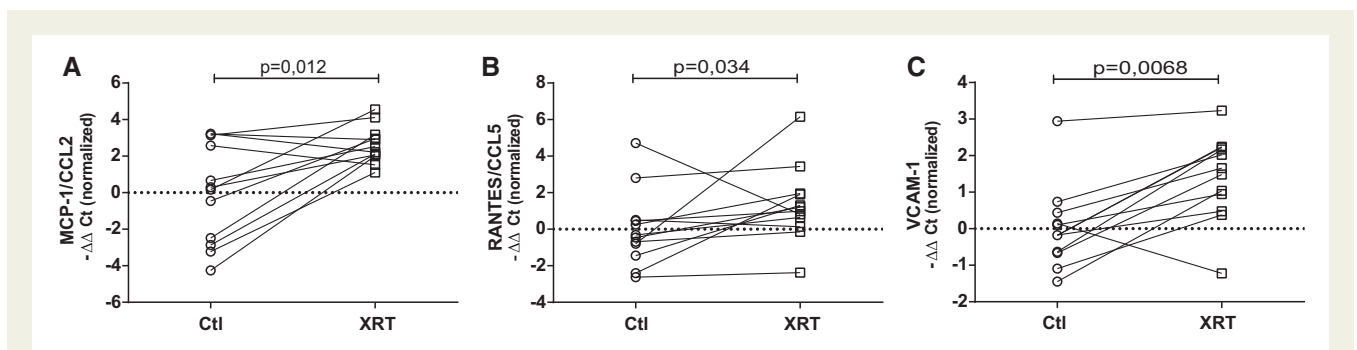
To investigate the chronic effects of radiotherapy on arterial gene expression, irradiated and non-irradiated human arteries from the same person were obtained from 10 patients and analysed by global gene expression profiling. Median time from last radiation therapy session to biopsy was 156 weeks. Comparison of the whole transcriptome between irradiated and non-irradiated biopsies using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Supplementary material online) identified apoptosis and NOD-like receptor signalling pathways as the most differentially expressed (Supplementary material online, Figure S1). Additional analysis showed a marked elevation of genes encoding IL-1 $\alpha$  and IL-1 $\beta$ , caspase-1 and NLR family Pyrin domain-containing protein (NLRP) 3 (Supplementary material online, Figure S2). Accordingly, we next performed a targeted network analysis focused on inflammasome-related transcripts (Figure 1), which supported induction of inflammasome-related transcripts and macrophage infiltration.

### Increased macrophage infiltration in human arteries with chronic radiation injury

We next turned to TaqMan qPCR to investigate mRNA levels of chemokine (C-C motif) ligand (*CCL*) 2 and *CCL5* associated with inflammasome activation and vascular cell adhesion molecule-1 (VCAM1), in arterial biopsies from 12 patients with chronic radiation injury.



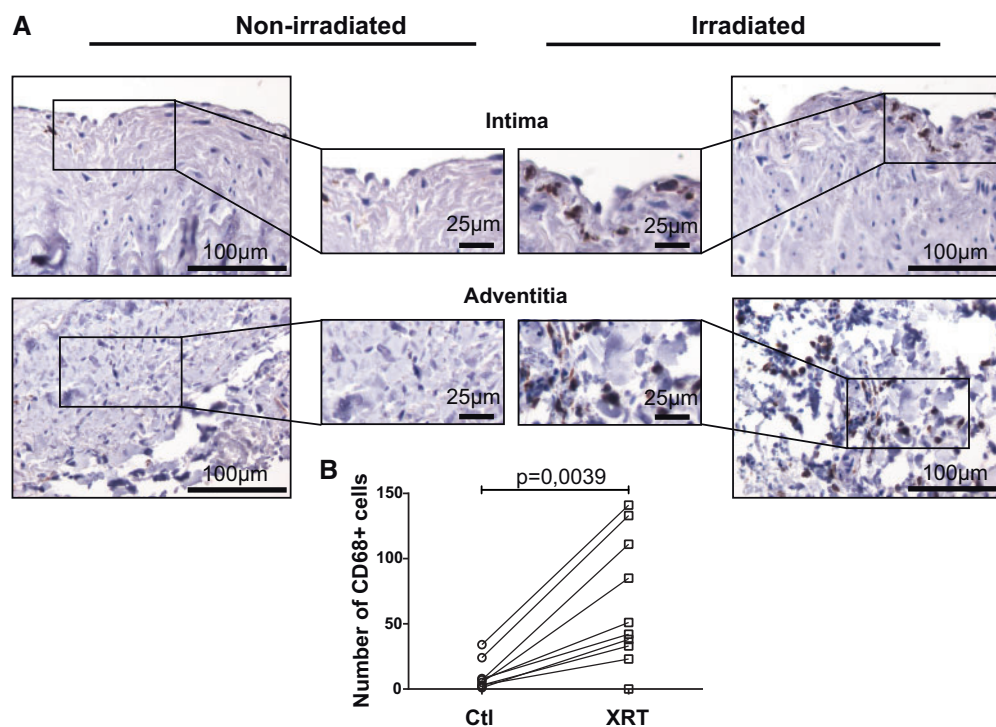
**Figure 1** Network of inflammasome-related transcripts in human arteries with or without chronic radiation injury. Biopsies of paired human arteries with and without chronic radiation injury were analysed using Affymetrix Gene Array ( $n = 20$ ) paired biopsies. (A) Inflammasome-related genes were mapped onto an interaction repository (BioGRID), a subnetwork extracted using the prize-collecting Steiner Forest graph optimization approach (PCSF) to include ‘bridges’ that connect these genes. Genes in the subnetwork are colour-coded to indicate the expression change between irradiated and non-irradiated. Genes associated with macrophages are shown on yellow background, endothelial cells (pink), and with T-cells/smooth muscle cells (grey). (B) Transcript levels of selected inflammasome associated genes. Centrelines correspond to median values. Hinges indicate the 25th and 75th percentiles. Whiskers indicate 1.5 times the interquartile range and observations beyond the whiskers are outliers. Difference between groups were analysed using the Wilcoxon signed rank test. CASP, caspase; CCL, chemokine (C-C motif) ligand; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; NLRP, NLR family Pyrin domain-containing protein.



**Figure 2** Inflammasome-associated transcripts were elevated in human arteries with chronic radiation injury. Biopsies of paired human arteries with and without chronic radiation injury were analysed using qPCR. mRNA levels of *CCL2/MCP-1* (A), *CCL5* (B), and *VCAM1* (C) in human arterial biopsies without (Ctl) and with (XRT) chronic radiation injury ( $n = 12$  patients) are plotted. Data are presented as  $-\Delta\Delta$ Ct normalized to the geometrical mean of the two endogenous controls. Differences between groups were analysed using the Wilcoxon signed ranked test. CCL, chemokine (C-C motif) ligand; MCP-1, monocyte chemoattractant protein-1; VCAM1, vascular cell adhesion molecule-1.

mRNA levels of all three genes were significantly elevated in arterial biopsies from irradiated compared with non-irradiated tissue (Figure 2). The marked elevation of *CCL2* in many irradiated biopsies suggests ongoing recruitment of inflammatory cells including monocytes/macrophages. Consequently, we stained for the macrophage

marker CD68. In paired irradiated and non-irradiated biopsies from 12 patients with a median time since last radiotherapy of 184 weeks. The number of CD68<sup>+</sup> cells was significantly higher in irradiated arteries (Figure 3), further supporting a continuing chronic innate immune response years after irradiation.



**Figure 3** Increased macrophage accumulation in human arteries with chronic radiation injury. Biopsies of paired human arteries with and without chronic radiation injury were sectioned and stained with anti-CD68 antibodies (brown) and haematoxylin QS. (A) Microscopic images of sections from biopsies with or without chronic radiation injury in one representative patient. Insets show higher magnification of the intima and adventitia layers, respectively. (B) Number of CD68<sup>+</sup> cells in sections of arterial biopsies ( $n = 12$ ). Differences between groups were analysed using the Wilcoxon signed ranked test. Ctl, non-irradiated; XRT, irradiated.

### Elevated levels of pro-caspase-1 and caspase-1 in human arteries with chronic radiation injury

To investigate whether the elevated levels of transcripts associated with inflammasome biology and macrophage infiltration observed in the irradiated arteries are linked to inflammasome activity, paired irradiated and non-irradiated biopsies from three patients in the BiRKA biobank were analysed for pro-caspase-1 and caspase-1. Western blot revealed that the irradiated arterial biopsies had a marked increase in pro-caspase-1 and caspase-1 (Figure 4, Supplementary material online, Figure S3) supporting increased inflammasome activity in chronic radiation injury.

### Mouse model of chronic radiation injury in the upper chest

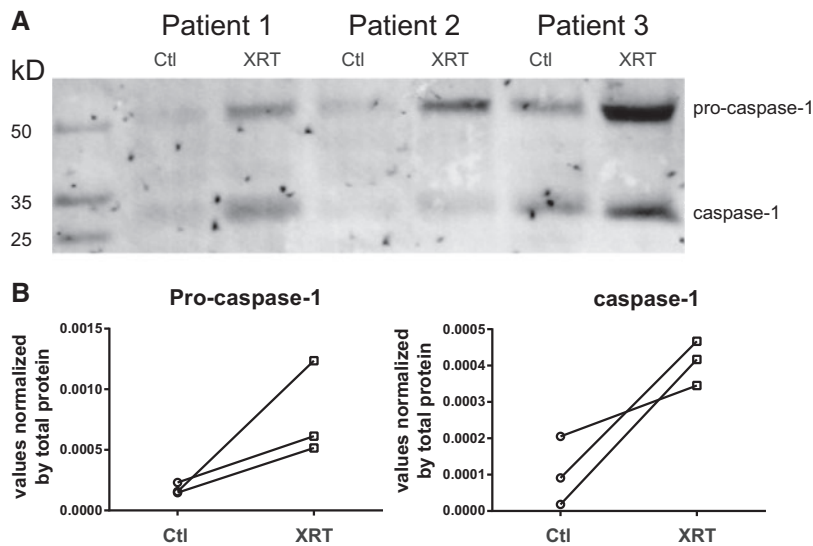
In light of the elevated caspase-1 protein and significantly elevated mRNA levels of caspase-1, NLRP3, IL-1 $\alpha$ , and IL-1 $\beta$  observed in human arterial biopsies here, we hypothesized that anakinra, modified recombinant IL-1Ra, attenuates arterial injury after radiotherapy. To test this, we used a model of radiation injury: female *Apoe*<sup>-/-</sup> mice were subjected to a controlled radiation injury or sham treatment with or without a subsequent 2 weeks of daily i.p. injections of anakinra. Tissues were harvested 10 weeks after radiation treatment (Figure 5A). Selective irradiation of the upper chest

and neck area resulted in a mean dose of 14 Gy in the target field, while surrounding tissue (5 mm) received a mean dose of 350 mGy (Figure 5B).

### Treatment with anakinra had no significant effects on body weight, blood lipids, collagen content in lesion, or atherosclerotic lesion size in irradiated *Apoe*<sup>-/-</sup> mice

Four groups of ten *Apoe*<sup>-/-</sup> mice were included (Figure 5A). Non-irradiated animals on average gained 0.4 g/week of body weight. There was no significant difference in weight gain between anakinra-treated and NaCl-treated mice in the non-irradiated mice. Irradiated animals lost an average of 0.25 g/week (Supplementary material online, Table S3). Plasma cholesterol levels were significantly increased in irradiated animals, regardless of anakinra-treatment, when compared with non-irradiated animals (Supplementary material online, Table S4). There was no significant difference in plasma triglyceride levels between irradiated and non-irradiated animals (Supplementary material online, Table S4).

Atherosclerotic lesion development was assessed both in sections of the aortic root and in *en face* preparations of the aortic arch and innominate artery. There was no significant difference between the four experimental groups in atherosclerosis lesion size at the aortic



**Figure 4** Elevated, pro-caspase and caspase-1 in human arteries with chronic radiation injury. Protein from paired human arteries with and without chronic radiation injury was analysed for procaspase-1 and caspase-1 using western blot ( $n = 3$ ) (A and C). Densitometry was measured using the image studio software and a Li-Cor scanner, normalized to total protein content, grouped by absence (Ctl) or presence (XRT) of chronic radiation injury, and plotted (B). kD, kilodalton.

root (Supplementary material online, Figure S4). There was no significant difference of atherosclerotic lesion size at the aortic arch between irradiated and non-irradiated mice, but atherosclerotic lesion size was reduced in the innominate artery in irradiated mice (Supplementary material online, Table S5). Lesion size in the aortic arch and innominate artery were not significantly different between anakinra-treated and NaCl-treated irradiated mice (Supplementary material online, Table S5). In non-irradiated mice, treatment with anakinra significantly increased atherosclerosis lesion size in the aortic arch, but not in the innominate artery (Supplementary material online, Table S5). There was no significant difference in collagen content between irradiated and sham treated mice (Supplementary material online, Figure S5). In line with the aortic root lesion size results, there was no significant difference between the four groups in measurements of the residual lumen volume and circumference of the artery at the aortic root as described by Alexander *et al.*<sup>15</sup>

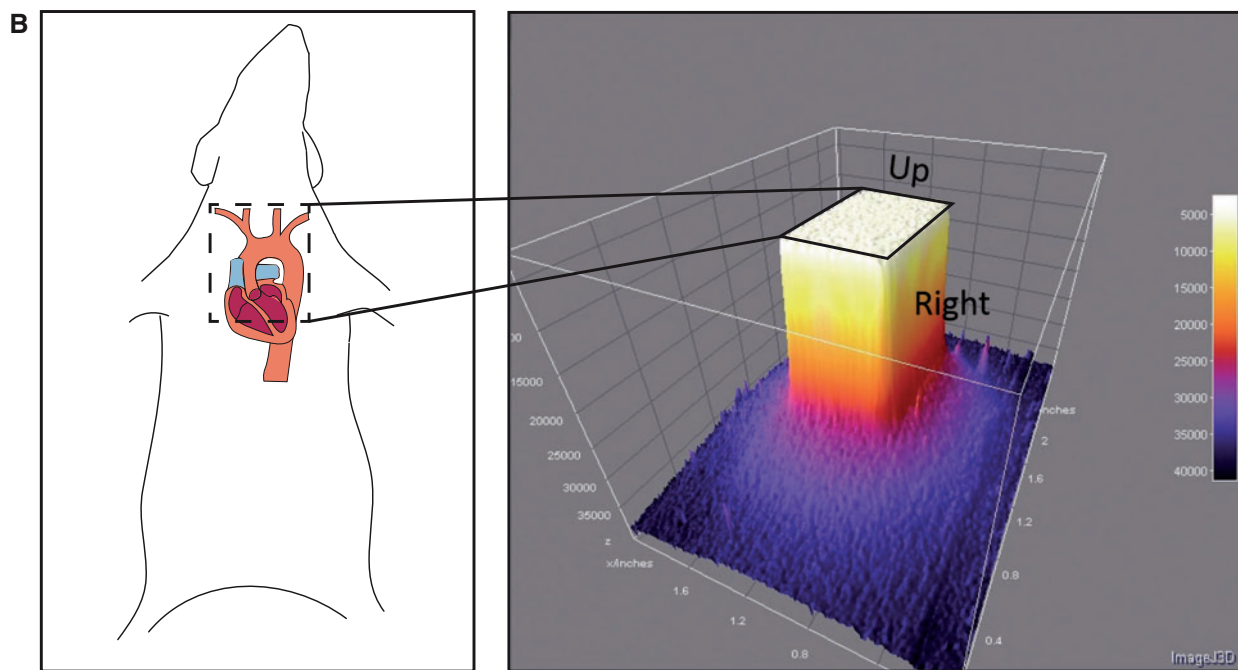
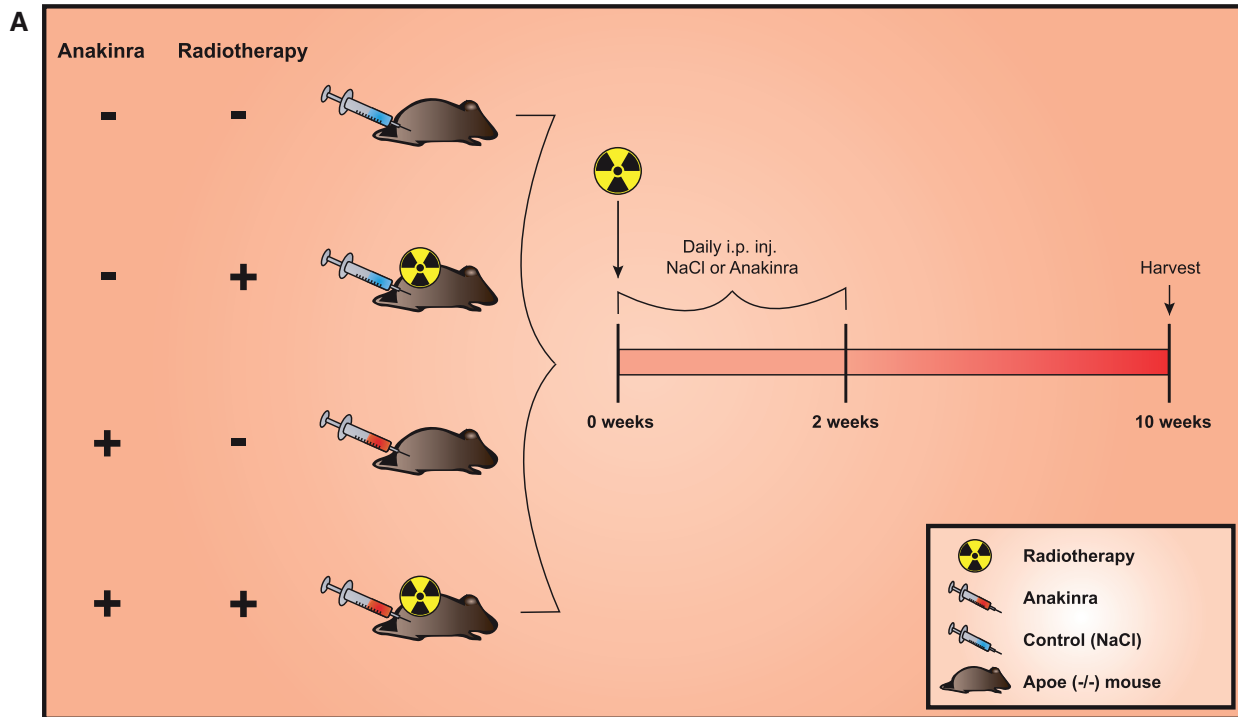
### Treatment with anakinra reduced arterial inflammation in irradiated *Apoe*<sup>-/-</sup> mice

Published data<sup>8,10</sup> and observations in the whole transcriptome comparison of human arteries with and without chronic radiation injury here identified transcripts associated with inflammation (Figure 1). Accordingly, *Ccl2*, *Ccl5*, *Vcam-1*, and *I-A<sup>b</sup>* Major Histocompatibility Class (MHC) II antigen were measured in arterial biopsies from all experimental animals included at the 10-week time point. Mean levels of *Ccl2* mRNA and the fraction of *I-A<sup>b+</sup>* area in the aortic root were significantly higher in samples from irradiated than in samples from non-irradiated mice (Figure 6A and D), indicating increased inflammatory activity in the irradiated compared with the non-irradiated arterial biopsies. Mean levels of *Ccl5* mRNA and fraction of *VCAM-1<sup>+</sup>*

area in aortic roots were higher in biopsies from irradiated mice, but the differences did not reach statistical significance (Figure 6B and C). Levels of pro-inflammatory cytokines in plasma were low as measured by mesoscale, supporting that significant systemic inflammation is limited in this model. There was no significant difference in the fraction of *CD68<sup>+</sup>* area in the aortic root lesions between radiated and non-irradiated mice (Supplementary material online, Figure S6A-B). However, 2 weeks of treatment with anakinra *i.p.* reduced the *CD68<sup>+</sup>* area in the aortic root lesions in control mice without altering the macrophage M1 and M2 subtype markers (Supplementary material online, Figure S6C-F). Anakinra-treatment of irradiated mice significantly attenuated the radiation-induced elevation of *Ccl2* and *I-A<sup>b</sup>* in arterial biopsies (Figure 6A and D) and significantly reduced levels of *Ccl5* mRNA together with a trend towards a reduced fraction of *VCAM-1<sup>+</sup>* area in aortic roots in irradiated mice (Figure 6B and C). Hence, anakinra-treatment significantly attenuated radiation-induced arterial inflammation as measured by *Ccl2*, *Ccl5*, and *I-A<sup>b</sup>*.

### Discussion

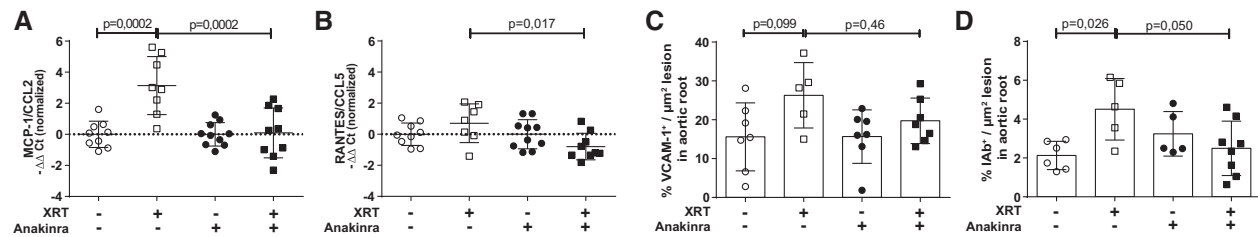
Paired irradiated and non-irradiated human arterial biopsies, collected during microvascular autologous free tissue transfer for cancer reconstruction in the same patient, enabled indepth analysis of chronic radiation-induced arterial damage. Elevated levels of inflammasome-related transcripts and caspase-1 was observed in arterial biopsies at a mean of 3 years after radiation exposure. Treatment of chronic radiation injury in mice with the IL-1Ra anakinra significantly reduced inflammation in arteries as evidenced by significant attenuation of *Ccl2* and *Ccl5* transcripts, and *I-A<sup>b</sup>*. Although no significant differences were observed between M1/M2



**Figure 5** A mouse model of anti-inflammatory treatment in upper chest and neck irradiation. (A) Overview of treatment groups, study timeline and treatment protocol. (B) Schematic illustration of the irradiation field in terms of anatomical overview (left), irradiation dose, distribution field, and radiation scatter (right). The X- and Z-axes show distance from the collimator in inches, and the Y-axis shows arbitrary units of radiation exposure. Colours illustrate the dose, ranging from yellow = high (14 Gy) to blue = low (non-detected). *Apoe*<sup>-/-</sup>, apolipoprotein E deficient; i.p; inj, intraperitoneal injection; NaCl, sodium chloride; +/-, received/did not receive treatment.

macrophage subtypes, the I-A<sup>b</sup> MHC class II antigen, a marker expressed on many pro-inflammatory macrophages<sup>9</sup> was up-regulated in irradiated arteries and subsequently decreased by

anakinra treatment. These data suggest that inhibiting IL-1 receptor signalling might be useful in counteracting long-term arterial adverse effects of radiotherapy.



**Figure 6** Treatment with anakinra reduced arterial inflammation in irradiated *Apoe*<sup>-/-</sup> mice. Female *Apoe*<sup>-/-</sup> mice were subjected to either a controlled radiation injury or sham treatment with or without a subsequent 2 weeks of daily i.p. injections of anakinra. Tissues were harvested and evaluated at 10 weeks after radiation treatment (A–D). RNA was isolated from thoracic aorta, and levels of *Ccl2/Mcp-1* (A) and *Ccl5* (B) analysed by qPCR. Aortic roots were cryosectioned and stained for VCAM-1 (C) and I-A<sup>b</sup> (MHC class II) (D). The stained area was measured in a section at 250/260 μm (VCAM-1/I-A<sup>b</sup> (MHC class II)) from the aortic valve plane and normalized to lesion area. Number of animals per group: Ctl = 9/7/6, XRT = 8/5/5, Ctl/Ak = 10/7/5, XRT/Ak = 9/7/8 for mRNA/VCAM/I-A<sup>b</sup> (MHC class II). Differences between groups were analysed using one-way ANOVA followed by Tukey *post hoc* analysis. *Ccl*, chemokine (C-C motif) ligand; Ctl, non-irradiated; *Mcp-1*, monocyte chemoattractant protein-1; MHC class II, major histocompatibility complex class II; VCAM-1, vascular cell adhesion molecule-1; XRT, irradiated.

The BiRka biobank enables paired analysis of arteries with and without chronic radiation injury simultaneously harvested in the same patient. Analysis of the whole transcriptome by Affymetrix gene array in a subset of these patients revealed that genes related to pathways involved in apoptosis and NOD-like receptor signalling were elevated in arteries with chronic radiation injury. This is interesting, since the mechanisms that underlie the pathogenesis of chronic arterial radiation injury, which can be a debilitating condition that contributes to a wide range of further complications, are not fully known. Epidemiological studies have reported that radiotherapy increases the risk of CVD at the site of irradiation many years after exposure,<sup>4,5</sup> but preventive therapy is lacking. It is, therefore, of key interest to map key molecular mechanisms involved in the chronic pathophysiology of arterial radiation injury.

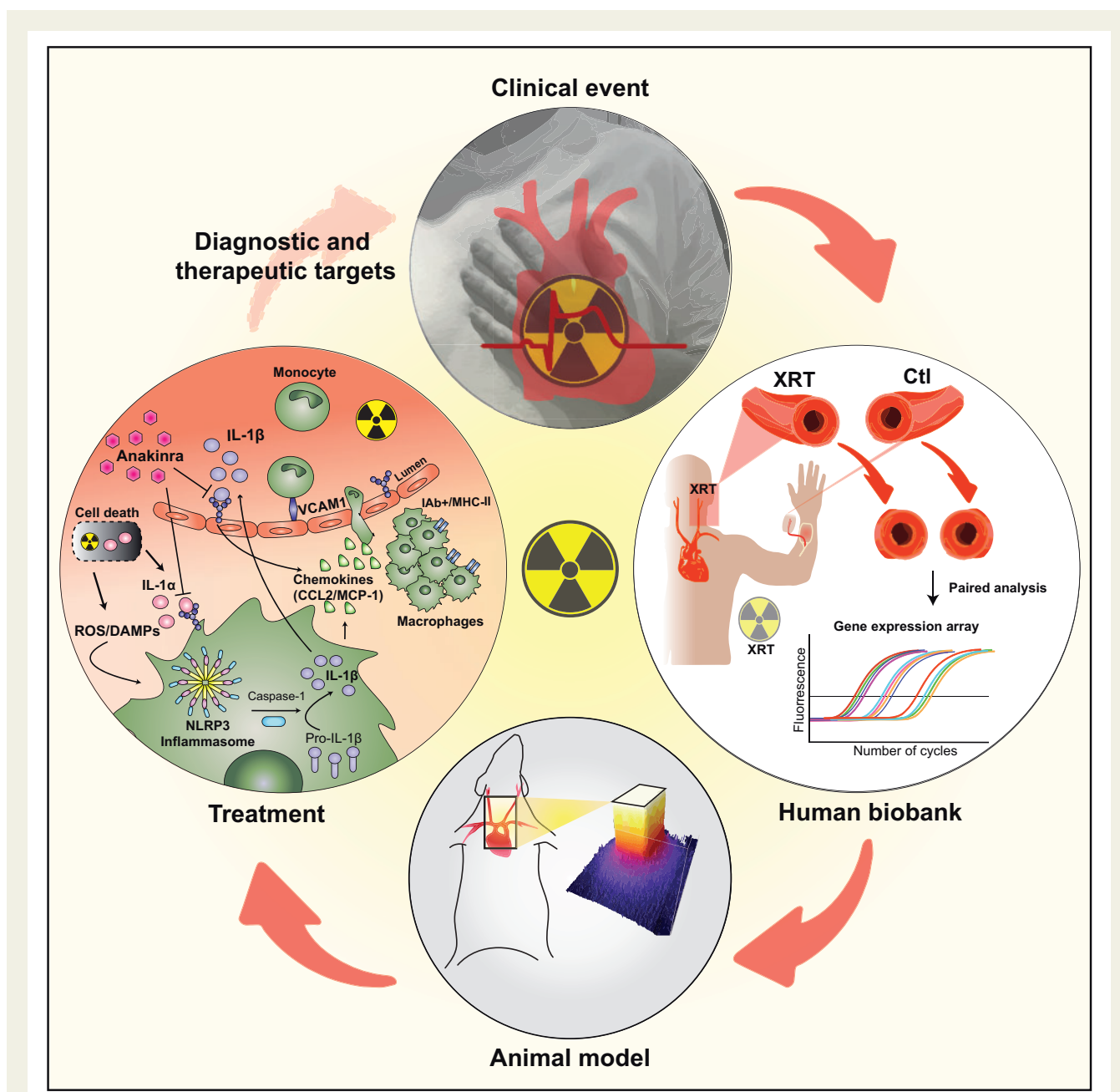
We have previously shown sustained inflammation in irradiated human arteries,<sup>16</sup> with activation of NF-κB and up-regulation of IL-1β after radiotherapy.<sup>8,10</sup> Elevation of *CCL2* was observed in the acute phase after radiotherapy in endothelial cells<sup>3</sup> and was also linked to radiation-induced CVD in a computational model.<sup>18</sup> The findings in the present study corroborate these observations in a more chronic human cohort. The median time since the last radiation therapy of the patients sampled for gene expression analysis was 156 weeks, i.e. 3 years, which is considerably longer than previous reports.<sup>14</sup> Given the long time since radiotherapy exposure in these patients, the sustained elevation of inflammasome-associated transcripts, including caspase-1 and NLRP3, together with the inflammasome product IL-1 and the downstream signalling transcripts *CCL2* observed here is particularly noteworthy. Together with increased protein levels of caspase-1 and an increased number of CD68<sup>+</sup> macrophages in arteries with chronic irradiation injury, these observations suggest on-going local inflammasome activity with chemokine production, and adhesion-molecule.

To study IL-1 blockade in radiation induced arterial disease, we used a mouse model that exhibits important features of human arteries with chronic radiation injury. Based on available data on human arteries with chronic radiation injury, including the

observations in the BiRka biobank here, we hypothesized that inhibition of IL-1 receptor activity would reduce inflammation in arteries with chronic radiation injury: Elevated levels of the ligand IL-1β increases expression of chemokines and adhesion molecules involved in monocyte recruitment to the arterial wall.<sup>19</sup> *CCL2* and *CCL5* are chemokines also associated with inflammasome activation that have been linked to CVD progression where therapeutic targeting is cardio-protective.<sup>20,21</sup> Anti-IL-1 therapy is already a promising candidate for treatment of CVD<sup>12–14,22,23</sup> and drugs that target IL-1 are available, e.g. the recombinant modified IL-1Ra ‘anakinra’ and the monoclonal antibody targeted at IL-1β ‘canakinumab’.<sup>24</sup> We used anakinra since both IL-1α and IL-1β levels were elevated in the transcriptome analysis. Treatment of irradiated *Apoe*<sup>-/-</sup> mice with anakinra returned levels of *Ccl2*, *Ccl5*, and I-A<sup>b</sup> in arteries with chronic inflammation to levels similar or equal to non-irradiated animals. Thus, treatment with anakinra abolished the radiation-induced elevation of these molecular markers of inflammation.

Previous studies have shown smaller, less stable, atherosclerotic lesions characterized by macrophage accumulation, decreased collagen and signs of intra-plaque haemorrhage in *Apoe*<sup>-/-</sup> mice subjected to irradiation.<sup>2,25</sup> Here, macrophage accumulation was observed in human arteries with chronic radiation injury. Furthermore, anakinra reduced CD68<sup>+</sup> area in aortic root lesions in mice, without any impact on the lumen size or vessel area in the aortic root. Interestingly, Choudhury *et al.*<sup>26</sup> showed that inhibition of IL-1 in age-related atherosclerosis reduced markers of inflammation, but does not affect the measures of vascular structures.

The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) showed that treatment with canakinumab significantly reduced the risk of secondary cardiovascular events in patients with previous myocardial infarction.<sup>13,27</sup> Interestingly, the CANTOS trial provided evidence that canakinumab treatment significantly reduces the incidence of and mortality in lung cancer.<sup>28</sup> This prospect becomes particularly intriguing considering that long-term cancer survivors are more likely to die from CVD than from malignancies.<sup>29</sup> Early prevention of late cardiovascular adverse effects is, therefore,



**Take home figure** A translational model for therapeutic target discovery in radiation injury. Clinical event: Increased risk for cardiovascular disease at the site of previous radiotherapy. Human biobank: Gene expression array of paired (irradiated and non-irradiated) human arteries retrieved at the same time during microvascular autologous free tissue transfers for reconstructive cancer surgery. Radiotherapy induces a sustained inflammatory response in human arteries. Animal model: Establishment of an animal model of local irradiation with similar inflammatory patterns as observed in the human biobank. Treatment: Treatment with anakinra (IL-1R antagonist) reduced radiation-induced vascular inflammation in our *Apoe*<sup>-/-</sup> mouse model of local irradiation. Ctl, non-irradiated; *Ccl2/Mcp-1*, chemokine (C-C motif) ligand2/monocyte chemoattractant protein-1; DAMP, damage associated molecular pattern; IL-1R, interleukin-1 receptor; ROS, reactive oxygen species; VCAM1, vascular cell adhesion molecule-1; XRT, irradiated. Illustration by Tinna Christersdottir based on published data.

highly desirable, provided it does not compromise the anti-tumour therapy. Safety studies of anakinra in rheumatoid arthritis patients have not shown any significant differences in cancer outcomes<sup>30</sup> and anakinra and canakinumab have been suggested as possible anti-cancer therapies.<sup>28,31,32</sup> Importantly, the cancer-related outcome

needs to be interpreted with caution since neither of these studies were originally designed for an oncology and properly designed clinical trials are needed. Further safety studies are therefore needed to evaluate the potential for IL-1 blockade in prevention of radiotherapy-induced CVD for cancer survivors.



## Temporal aspects of treatment against radiation induced cardiovascular disease

Radiation injury has acute and late adverse effects.<sup>1</sup> Our data support that early inhibition of acute innate immune response following radiotherapy treatment may lead to decreased infiltration of immune cells into the vessel wall and reduced chronic inflammation. In the CANTOS trial, patients with a history of myocardial infarction and elevated CRP resumed treatment with canakinumab for a median time of 3.7 years, where treatment with 150 mg canakinumab showed reduced number of cardiovascular events.<sup>13</sup> Smaller studies have used anakinra for shorter treatment periods down to 2 weeks with a positive effect on cardiovascular outcome.<sup>22,33</sup> The rationale for the 2 weeks treatment in the current context was to test the hypothesis that early inhibition of acute innate immune response following radiotherapy could ameliorate late adverse effects.<sup>17,32</sup>

## Study limitations

The vessel length that can be collected from each patient without compromising the microvascular surgery is limited and biopsy for both mRNA and protein analysis in the same patient was not possible. Arterial biopsies years after radiotherapy exposure was not possible in mice, given their life span. However, the vascular inflammation in *ApoE*<sup>-/-</sup> mice at 20 weeks of age was similar to the human inflammatory phenotype. Atherosclerotic lesions are generally observed in *ApoE*<sup>-/-</sup> mice at this age independently of radiation. However, radiation appears to cause more of a general sterile inflammation of the vessel wall, i.e. arteriosclerosis, rather than atherosclerosis.<sup>16</sup> This phenotype limited the usefulness of atherosclerosis as an endpoint in the present context. Consequently, this study focused on arteriosclerosis and vascular inflammation rather than atherosclerosis development.

## Conclusion

Persistent vascular inflammation after radiotherapy is a clinical problem for cancer survivors that can promote CVD. The present study shows elevated levels of molecules associated with inflammasome activation and vascular inflammation, including *NLRP3*, *CASP1*, *CCL2*, *CCL5*, *VCAM1*, and *CD68* in human arteries with chronic radiation injury. Atherosclerosis-prone *ApoE*<sup>-/-</sup> deficient mice irradiated in the neck/chest region showed increased local vascular inflammation weeks after radiation exposure. Treatment of irradiated mice with anakinra significantly reduced *Cd2* and *Cd5* mRNA levels and I-A<sup>b</sup> MHC class II antigen in the aorta. Thus, Anakinra administered directly after radiation exposure for 2 weeks, ameliorated the sustained radiation-induced expression of inflammatory mediators in mice (*Take home figure*). Further studies are warranted to evaluate IL-1 blockade as a potential treatment of radiotherapy-induced vascular disease in a clinical setting when the effects on cancer patients has been evaluated.

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

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**Conflict of interest:** The pharmaceutical company Swedish Orphan Biovitrum International AB provided drug Kineret<sup>®</sup> (anakinra).

## References

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