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FASTTRACK CLINICAL RESEARCH
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Modulation of the interleukin-6 signalling pathway and incidence rates of atherosclerotic events and all-cause mortality: analyses from the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS)

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Aims Canakinumab, a monoclonal antibody targeting interleukin (IL)-1β, reduces rates of recurrent cardiovascular events without lowering lipids. It is uncertain, however, to what extent these beneficial cardiovascular outcomes are mediated through interleukin-6 (IL-6) signalling, an issue with substantial pathophysiologic consequences and therapeutic implications.

Methods and results A total of 4833 stable atherosclerosis patients in the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) had IL-6 levels measured before randomization and after treatment with placebo or one of three doses of canakinumab (50 mg, 150 mg, or 300 mg) given subcutaneously once every 3 months. Participants were followed for up to 5 years (median follow-up 3.7 years). Compared with those allocated to placebo, CANTOS participants receiving canakinumab who achieved on-treatment IL-6 levels below the study median value of 1.65 ng/L experienced a 32% reduction in major adverse cardiovascular events [MACE, multivariable adjusted hazard ratio (HR^{adj}) 0.68, 95% confidence interval (CI) 0.56–0.82; $P < 0.0001$], a 30% reduction in MACE plus the additional endpoint of hospitalization for unstable angina requiring urgent revascularization (MACE+, HR^{adj} 0.70, 95% CI 0.59–0.84; $P < 0.0001$), a 52% reduction in cardiovascular mortality (HR^{adj} 0.48, 95% CI 0.34–0.68; $P < 0.0001$), and a 48% reduction in all-cause mortality (HR^{adj} 0.52, 95% CI 0.40–0.68; $P < 0.0001$) with prolonged treatment. In contrast, those with on-treatment IL-6 levels equal to or above 1.65 ng/L after taking the first dose of canakinumab had no significant benefit for any of these endpoints. These differential findings based on the magnitude of IL-6 response were seen in analyses alternatively based on tertiles of on-treatment IL-6 levels, and in analyses using a statistical inference approach to estimate the effect of treatment among individuals who would achieve a targeted IL-6 level.

Conclusion CANTOS provides proof of concept evidence in humans that modulation of the IL-6 signalling pathway, at least with canakinumab, associates with reduced cardiovascular event rates, independent of lipid lowering.

Clinical trial registration ClinicalTrials.gov NCT01327846.

Keywords Inflammation • Interleukins • Clinical trial • Myocardial infarction

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Introduction

The central pleiotropic inflammatory cytokine interleukin-6 (IL-6) can affect multiple cell types mediated either through binding to the membrane bound IL-6 receptor (classical IL-6 signalling) or through binding to soluble IL-6 receptors (IL-6 trans-signalling). Interleukin-6 participates in the pathogenesis of multiple inflammatory disorders and inhibitors of IL-6 signalling are commonly used to treat rheumatoid arthritis and other classical inflammatory disorders such as Crohn's disease and psoriasis.[1]

Nearly 20 years ago, we demonstrated that plasma IL-6 levels were powerful predictors of future vascular events, independent of traditional risk factors.[2,3] Prospective cohorts have replicated these results[4–6] and extended them to risks of all-cause mortality among patients with unstable coronary syndromes.[7,8] Further, Mendelian randomization studies support a potential causal role for the IL-6 signalling pathway in atherothrombosis.[9,10] To date, however, no clinical outcome data have been available relating IL-6 reduction to vascular event reduction.

The Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) demonstrated that canakinumab, a human therapeutic monoclonal antibody targeting interleukin-1β, significantly reduces major adverse cardiovascular event (MACE) rates in the absence of any lipid lowering effects.[11] CANTOS also showed that the cardiovascular benefits of canakinumab are greatest among those who achieve the greatest reduction in C-reactive protein (hsCRP), a downstream biomarker of the acute phase response.[12] Interleukin-1β, the target of canakinumab, strongly induces IL-6 production by many cell types including vascular endothelial and smooth muscle cells.[13,14] Yet, whether the benefits of canakinumab treatment involve the IL-6 signalling pathway remains uncertain. This issue has substantial pathophysiologic consequences and therapeutic implications.

To address these issues, we conducted a sub-study among 4833 trial participants designed to address the relationship of IL-6 reduction to cardiovascular event reduction in CANTOS. Specifically, we assessed the effects of canakinumab on rates of MACE, cardiovascular mortality, and all-cause mortality occurring with long-term treatment over a maximum follow-up period of 5 years in CANTOS according to on-treatment levels of IL-6 achieved measured 3 months into the trial, just before administration of the second dose of randomized study treatment.

Methods

Patients and trial design

CANTOS, a randomized, double-blind placebo controlled trial, evaluated three doses of canakinumab (50 mg, 150 mg, or 300 mg) given subcutaneously once every 3 months when compared with matching subcutaneous placebo for the prevention of atherosclerotic events.[11,15] Overall, CANTOS enrolled between April 2011 and March 2014, 10 061 patients with a history of prior myocardial infarction and levels of hsCRP greater than or equal to 2 mg/L from over 1000 clinical sites in 39 countries. The study excluded patients with a history of chronic or recurrent infections, previous malignancy other than basal cell skin carcinoma, a suspected or known immunocompromised state, or a history of (or at high risk for) tuberculosis or HIV-related disease, and those using systemic anti-inflammatory treatments. All trial participants provided written informed consent to participate in the trial, which was overseen by an independent data and safety monitoring board. The trial primary endpoint was a composite of adjudicated recurrent myocardial infarction, stroke, or cardiovascular death (MACE). The key pre-specified secondary cardiovascular efficacy endpoint included these events and adjudicated episodes of hospitalization for unstable angina requiring urgent coronary revascularization (MACE+). Additional major endpoints adjudicated by the trial endpoint committee included cardiovascular mortality and all-cause mortality. Median follow-up was 3.7 years.

Measurement of interleukin-6

Interleukin-6 levels were evaluated at baseline and 3 months after randomization in a subset of 4833 CANTOS participants; these individuals did not differ from those enrolled in CANTOS as a whole except that those in the IL-6 substudy were predominantly enrolled at clinical sites in North American and Western Europe. All IL-6 assays were performed on plasma collected in EDTA in a central laboratory using the Quantikine high sensitivity enzyme-linked immunosorbent assay (ELISA) for human IL-6 (R & D Systems, catalogue number HS600B, Minneapolis MN, USA) for which intra-assay and inter-assay coefficients of variation range from 6.5% to 9.5%.

Statistical analysis

On an *a priori* basis, we divided trial participants allocated to canakinumab into two groups according to whether the level of IL-6 was less than, or equal to or greater than 1.65 ng/L at 3 months, the median on-treatment value for those allocated to active therapy after receiving the first dose of treatment. We then used Cox proportional-hazards models stratified by time since index myocardial infarction to estimate relative hazards for MACE, cardiovascular mortality, and all-cause mortality in these two groups, compared with those allocated to placebo. *P*-values for the test of trend were calculated across these three groups scored as 0, 1, or 2, and the Kaplan–Meier curves were evaluated visually for differences between groups.

Although conducted within the context of a randomized trial, on-treatment analyses are inherently observational. Thus, several methods were used to address issues of potential confounding and to ensure that results were consistent using alternative cut-points for on-treatment levels of IL-6.

First, multivariable modelling was used to adjust for baseline characteristics including age, gender, smoking status, hypertension, diabetes, and body mass index, and baseline levels of LDL cholesterol and IL-6. This analysis allows for a comparison of univariate hazard ratios (HR) to multivariate HRs as a method to address the magnitude of potential confounding in achieved IL-6 levels that may be due to baseline differences between participant characteristics, rather than to direct effects of canakinumab.

Second, to assess the impact of different on-treatment thresholds, we repeated the above univariate and multivariate analyses using on-treatment tertiles of IL-6 at 3 months (rather than on-treatment levels above or below the study median).

Third, as an internal check on the validity of our data and to assess the magnitude of IL-6 change rather than achievement of any specific threshold value, we repeated the above analyses on the basis of achieving reductions in IL-6 of greater than or less than 50%.

Fourth, we conducted separately a statistical inference analysis, which compared potential outcomes of individual canakinumab treated participants had they counterfactually been treated with placebo. In particular, we conducted this later analysis to those assigned to 150 mg canakinumab once every 3 months, the canakinumab dose regimen being evaluated by regulatory authorities for use in atherosclerosis prevention. Details of this analysis are contained in the Supplementary material online.

All *P*-values are two-sided, and all confidence intervals (CIs) computed at the 95% level.

Table 1 Baseline clinical characteristics of the CANTOS population in the placebo group and in the canakinumab groups according to achieved concentrations of interleukin-6 above or below the median 3 month on-treatment value of 1.65 ng/L

Baseline characteristics	Placebo ($n = 1597$)	Canakinumab, 3 month on-treatment Il-6 above the median value (≥1.65 ng/L) ($n = 1619$)	Canakinumab, 3-month on-treatment IL-6 below the median value (<1.65 ng/L) ($n = 1617$)
Age (years)	62.0 (55.0–69.0)	64.0 (57.0–70.0)	60.0 (53.0–67.0)*
Female sex	413 (25.9)	399 (24.6)	417 (25.8)
Region			
Asia	96 (6.0)	95 (5.9)	94 (5.8)*
Central Europe	260 (16.3)	276 (17.1)	271 (16.8)
Latin America	110 (6.9)	126 (7.8)	72 (4.5)
North America	587 (36.8)	555 (34.3)	632 (39.1)
Western Europe	544 (34.1)	567 (35.0)	548 (33.9)
Current smoking	397 (24.9)	486 (30.0)	389 (24.1)*
Body mass index (kg/m^2)	30.4 (27.0–34.9)	30.8 (27.2–35.1)	30.1 (24.1–33.9)*
Waist circumference (cm)	106.0 (97.0–117.0)	107.3 (98.1–118.0)	105.0 (96.0–114.3)*
Hypertension	1285 (80.5)	1365 (84.3)	1284 (79.4)*
Diabetes	681 (42.6)	740 (45.7)	586 (36.2)*
Qualifying myocardial infarction			
STEMI	791 (49.5)	811 (50.1)	855 (52.9)
Non-STEMI	574 (35.9)	571 (35.3)	556 (34.4)
Unknown/missing	232 (14.5)	236 (14.6)	206 (12.7)
History of PCI	1067 (66.8)	1096 (67.8)	1139 (70.4)
History of CABG	269 (16.8)	323 (20.0)	205 (12.7)*
History of congestive heart failure	306 (19.2)	390 (24.1)	249 (15.4)*
Lipid lowering therapy	1494 (93.6)	1500 (92.8)	1518 (94.1)
Renin-angiotensin inhibitors	1250 (78.6)	1304 (80.9)	1283 (79.6)
Anti-ischaemia agents*	1477 (92.5)	1503 (93.0)	1476 (91.5)
hsCRP (mg/L)	4.15 (2.80–6.85)	4.90 (3.15–8.20)	3.70 (2.55–5.90)*
Interleukin-6 (ng/L)	2.58 (1.78–4.03)	3.54 (2.43–5.50)	1.93 (1.45–2.63)*
Total cholesterol (mg/dL)	162.0 (139.6–189.0)	159.3 (134.6–187.9)	163.1 (140.9–191.0)*
LDL cholesterol (mg/dL)	83.9 (66.1–107.0)	82.0 (63.0–106.0)	84.0 (65.4–108.0)*
HDL cholesterol (mg/dL)	45.6 (38.7–53.8)	43.0 (36.4–52.0)	45.4 (38.0–54.0)*
Triglycerides (mg/dL)	138.1 (100.1–195.7)	136.4 (100.1–192.0)	139.1 (103.6–195.7)
eGFR (mL/min/1.73 m^2)	79.0 (65.0–93.0)	75.0 (60.0–90.0)	79.0 (67.0–93.0)*
Randomized to canakinumab 50 mg		617 (38.1)	401 (24.8)*
Randomized to canakinumab 150 mg		570 (35.2)	568 (35.1)
Randomized to canakinumab 300 mg		432 (26.7)	648 (40.1)

*$P < 0.05$.

Results

Table 1 shows baseline characteristics of the study population in the placebo group and in the combined canakinumab groups according to whether the on-treatment IL-6 level was below vs. at or above the median value of 1.65 ng/L when measured at 3 months (before receiving the next dose).

In the placebo group, consistent with prior cohort studies,[2–8] baseline levels of IL-6 associated with increased risk of future cardiovascular events; in this group, the relative risks (95% CI) for MACE across increasing tertiles of baseline IL-6 were 1.0 (referent), 1.15 (0.86–1.54), and 1.47 (1.11–1.94) (P-trend across tertiles = 0.007) whereas the comparable values for MACE+ were 1.0 (referent), 1.15 (0.87–1.52), and 1.40 (1.08–1.83) (P-trend across tertiles = 0.01). *Figure 1* presents incidence rates for MACE, MACE+, cardiovascular mortality, and all-cause mortality in the cohort as a whole stratified by baseline tertile of IL-6.

Overall, compared with placebo, canakinumab reduced IL-6 levels at 3 months by 34.9% ($P < 0.001$). These effects were dose-dependent such that the placebo-subtracted median percent reductions in IL-6 at 3 months were 24.5%, 35.8%, and 42.7% for the 50 mg, 150 mg, and 300 mg doses, respectively.

As would be anticipated, participants who subsequently achieved 3 month levels below 1.65 ng/L had lower baseline IL-6 levels

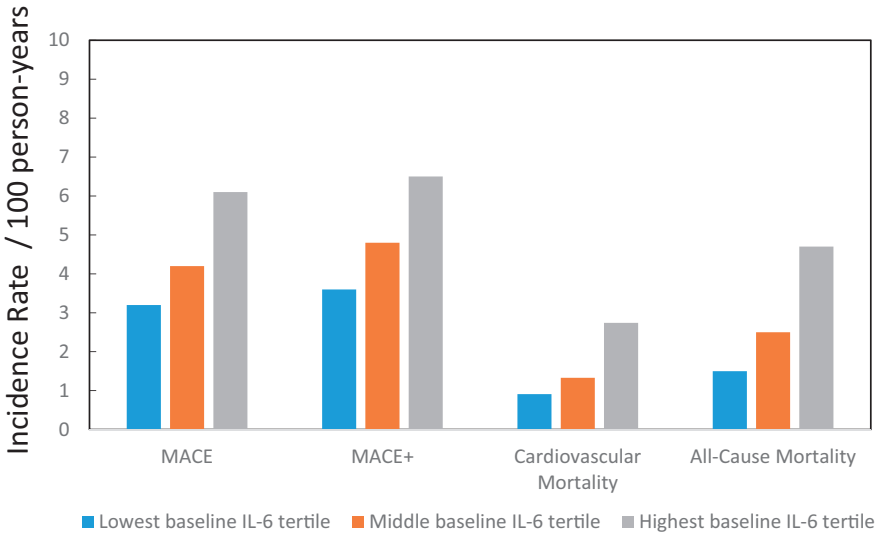
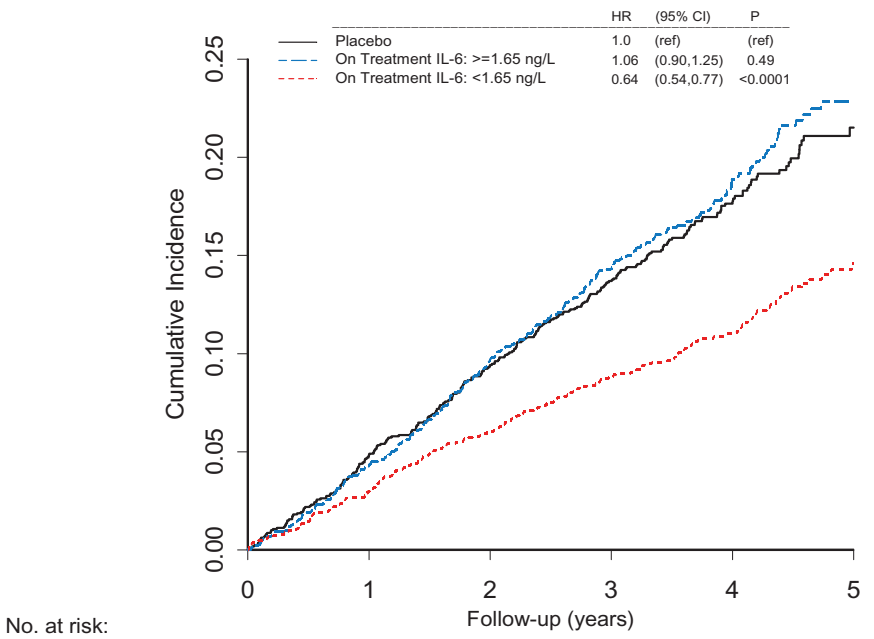


Figure 1 Incidence rates (per 100 person-years) for myocardial infarction, stroke and cardiovascular death (MACE), myocardial infarction, stroke, hospitalization for unstable angina requiring urgent revascularization, cardiovascular death (MACE+), cardiovascular mortality, and all-cause mortality in CANTOS according to increasing baseline tertiles of interleukin-6.

Table 2 Incidence rates (per 100 person years) and hazard ratios for cardiovascular endpoints and total mortality in CANTOS, according to the achievement of on-treatment interleukin-6 levels above or below the trial median of 1.65 mg/L at 3 months among those allocated to canakinumab

Treatment group, on-treatment IL-6 threshold	*N*	Incidence rate (*n*)[a]	HR (95% CI)	*P*-value	HR[adjusted] (95% CI)[b]	*P*-value
MACE						
Placebo	1597	4.91 (282)	1 (ref)		1 (ref)	
Canakinumab, IL-6 ≥ median value (1.65 mg/L)	1619	5.15 (291)	1.06 (0.90–1.25)	0.49	0.90 (0.76–1.07)	0.25
Canakinumab, IL-6 < median value (1.65 mg/L)	1617	3.21 (199)	0.64 (0.54–0.77)	<0.0001	0.68 (0.56–0.82)	<0.0001
P-value for trend across categories				<0.0001		<0.0001
MACE+						
Placebo	1597	5.49 (311)	1 (ref)		1 (ref)	
Canakinumab, IL-6 ≥ median value (1.65 mg/L)	1619	5.44 (305)	1.00 (0.85–1.17)	0.97	0.87 (0.74–1.02)	0.093
Canakinumab, IL-6 < median value (1.65 mg/L)	1617	3.72 (228)	0.67 (0.57–0.80)	<0.0001	0.70 (0.59–0.84)	<0.0001
P-value for trend across categories				<0.0001		<0.0001
Cardiovascular mortality						
Placebo	1597	1.66 (103)	1 (ref)		1 (ref)	
Canakinumab, IL-6 ≥ median value (1.65 mg/L)	1619	2.26 (136)	1.38 (1.07–1.79)	0.0134	1.15 (0.88–1.51)	0.30
Canakinumab, IL-6 < median value (1.65 mg/L)	1617	0.72 (47)	0.43 (0.30–0.60)	<0.0001	0.48 (0.34–0.68)	<0.0001
P-value for trend across categories				<0.0001		0.0002
Total mortality						
Placebo	1597	2.86 (177)	1 (ref)		1 (ref)	
Canakinumab, IL-6 ≥ median value (1.65 mg/L)	1619	3.91 (235)	1.39 (1.14–1.69)	0.0010	1.17 (0.96–1.44)	0.12
Canakinumab, IL-6 < median value (1.65 mg/L)	1617	1.33 (87)	0.46 (0.36–0.60)	<0.0001	0.52 (0.40–0.68)	<0.0001
P-value for trend across categories				<0.0001		<0.0001

[a]Per 100 person-years of exposure.
[b]Covariates included in the adjusted multivariable model include age, gender, smoking status, hypertension, diabetes, body mass index, baseline level of IL-6, and baseline level of LDL cholesterol.
The median percent reduction in IL-6 at 3 months was -34.9.



No. at risk:

Placebo	1597	1501	1411	1254	635	153
Canakinumab:						
IL-6 >=1.65 ng/L	1619	1524	1411	1211	562	123
IL-6 <1.65 ng/L	1617	1559	1501	1371	772	211

Figure 2 Cumulative incidence of myocardial infarction, stroke, and cardiovascular death (MACE) in CANTOS in the placebo group and in the combined canakinumab groups according to whether 3 months on-treatment IL-6 levels were above or below the on-treatment median value of 1.65 ng/L.

compared with those who did not. The proportions of individuals achieving on treatment IL-6 levels below the trial median was 39%, 50%, and 60% in the canakinumab 50 mg, 150 mg, and 300 mg groups, respectively ($P < 0.0001$).

In univariate analyses, the magnitude of IL-6 reduction at 3 months related directly to the magnitude of clinical benefit associated with continued canakinumab treatment. Compared with those allocated to placebo, CANTOS participants allocated to canakinumab who achieved on-treatment IL-6 levels below the median value of 1.65 ng/L at 3 months experienced a 36% reduction in MACE (HR 0.64, 95% CI 0.54–0.77; $P < 0.0001$). In contrast, those with on-treatment IL-6 levels equal to or above 1.65 ng/L after taking the first dose of canakinumab had no significant benefit reduction in MACE (HR 1.06, 95% CI 0.90–1.25; $P = 0.49$). These data correspond to incidence rates for MACE of 4.91, 5.15, and 3.21 events per 100 person years in the placebo group and in the combined canakinumab groups that did not and did achieve IL-6 levels below 1.65 ng/L, respectively (*Table 2* and *Figure 2*).

Similar findings were observed for the expanded MACE endpoint, cardiovascular mortality, and all-cause mortality. Specifically, compared with those allocated to placebo, CANTOS participants allocated to canakinumab who achieved on-treatment IL-6 levels below the median value of 1.65 ng/L at 3 months experienced a 33% reduction in expanded MACE (HR 0.67, 95% CI 0.57–0.80; $P < 0.0001$), a 57% reduction in cardiovascular mortality (HR 0.43, 95% CI 0.30–0.60; $P < 0.0001$), and a 54% reduction in all-cause mortality (HR 0.46, 95% CI 0.36–0.60; $P < 0.0001$) with prolonged treatment. In contrast, those with on-treatment IL-6 levels equal to or above 1.65 ng/L after taking the first dose of canakinumab derived no significant benefit for any of these endpoints (*Table 2* and *Figure 3*).

Virtually identical findings were observed in sensitivity analyses that excluded the small number of non-fatal cardiovascular events that occurred before 3 months of follow-up.

Several analyses evaluated the robustness of these findings and addressed whether confounding factors might have had magnitudes of effect on achieved IL-6 similar to that of canakinumab itself.

First, we simultaneously adjusted for baseline IL-6 and LDL cholesterol level, as well as for clinical characteristics known to impact IL-6 modestly (including age, gender, smoking status, hypertension, diabetes, and body mass index). In these multivariable analyses, the calculated HRs for MACE among those treated with canakinumab who had IL-6 levels at 3 months below or above 1.65 ng/L (adjusted HRs 0.68 and 0.90, respectively) changed minimally from those observed in our univariate analysis (unadjusted HRs 0.64 and 1.06, respectively). Minimal change in HRs following multivariable adjustment was also observed for expanded MACE, cardiovascular mortality, and all-cause mortality (*Table 2*). Taken together, the marginal change in HRs after multivariable adjustment suggest that the biologic effects of canakinumab on achieved IL-6 levels is both independent of and substantially greater than effects on achieved IL-6 levels associated with baseline characteristics of the study population.

Second, we repeated our analysis for each cardiovascular endpoint across tertiles of on treatment IL-6 levels (rather than median levels) at 3 months. Effects were again similar in both univariable and multivariable analyses with the greatest reductions in risk for all endpoints

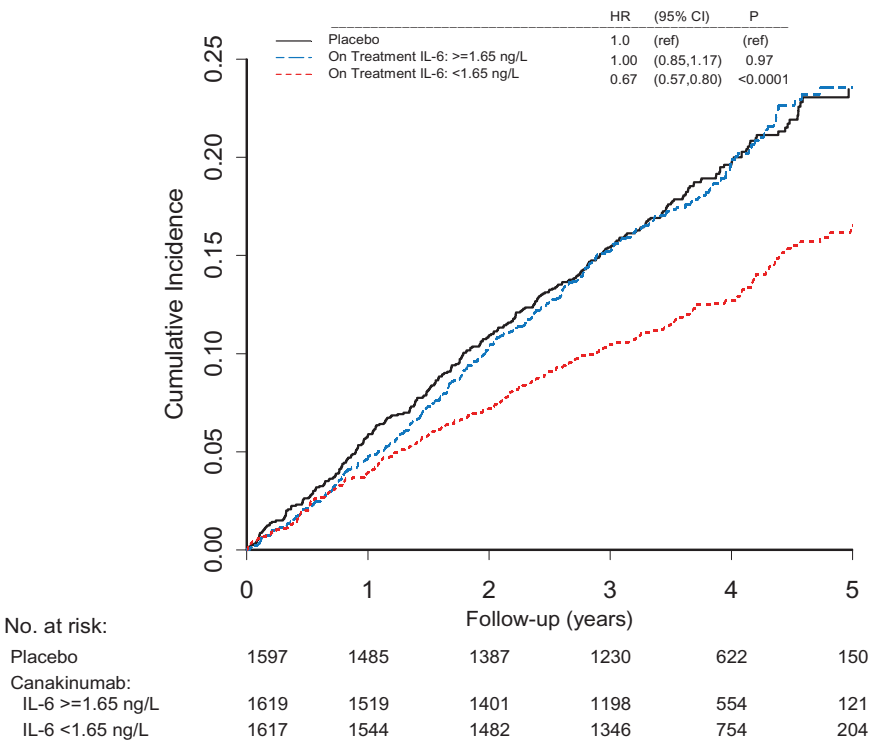


Figure 3 Cumulative incidence of myocardial infarction, stroke, hospitalization for unstable angina requiring urgent revascularization, and cardiovascular death (MACE+) in CANTOS in the placebo group and in the combined canakinumab groups according to whether 3 months on-treatment IL-6 levels were above or below the on-treatment median value of 1.65 ng/L.

accruing among those with the greatest magnitude of IL-6 reduction (*Table 3*). For example, the multivariable adjusted HRs for MACE were 1.0, 0.99, 0.73, and 0.65 for the placebo, top IL-6 tertile, middle IL-6 tertile, and lowest IL-6 tertile, respectively (*P*-value for trend across groups < 0.0001).

Third, a statistical inference analysis was conducted in which we modelled potential outcomes on placebo using baseline covariates (age, gender, body mass index, smoking status, diabetes, blood pressure, baseline hsCRP, total and HDL cholesterol, glomerular filtration rate, prior history and timing of vascular disease combined into a score and baseline IL-6, medical history of recurrent MI, presence of heart failure, non-HDL-C, systolic blood pressure, HbA1c) of individual canakinumab treated patients had they counterfactually been allocated to placebo, and then compared the modelled survival to observed survival. In this alternative analysis approach designed to address the estimation of treatment effect of canakinumab in patients who had achieved target levels of IL-6, we again saw highly similar results. For example, for those treated with 150 mg canakinumab who achieved a 3 months IL-6 below the trial median value of 1.65 ng/L, the HR for MACE compared with their outcome had they counterfactually been assigned to placebo was 0.64 (95% CI 0.47–0.84) whereas for those whose 3 months IL-6 was ≥1.65 mg/L, the comparable relative hazard was 0.95 (95% CI 0.75–1.17) (see Supplementary material online).

Similar differential effects, though somewhat attenuated, were observed in analyses of canakinumab treated patients who achieved 50% or greater reductions in IL-6 at 3 months when compared with those who did not. For example, compared with placebo, those treated with 150 mg canakinumab who achieved a greater than 50% reduction in IL-6 at 3-months had a relative HR for MACE of 0.74 (95% CI 0.55–1.00), whereas the comparable relative HR was 0.82 (95% CI 0.66–1.02) for those who achieved a less than 50% reduction in IL-6 (*P*-value for trend across treatment categories 0.02). Despite smaller sample size, similar differential effects according to percent reduction in IL-6 were also seen for the cardiovascular and all-cause mortality endpoints when comparing the total canakinumab group to placebo.

As previously described, canakinumab had no hepatic or renal toxicities, was associated with reduced rates of lung cancer and was neutral for all-cause mortality.[11,15] In contrast, canakinumab was associated with an increase in fatal infection that occurred in approximately one in every 1000 treated patients. This latter effect was neither dose-dependent nor related to achieved on-treatment levels of IL-6.

Discussion

In CANTOS as a whole, random allocation to canakinumab at doses of 150 mg or 300 mg once every 3 months reduced cardiovascular event rates by 15% for MACE ($P = 0.007$) and 17% for MACE+ ($P = 0.0006$) while the 50 mg dose had non-significant effects.[11] A

Table 3 Incidence rates (per 100 person years) and hazard ratios for cardiovascular endpoints and total mortality in CANTOS, according to on-treatment tertiles of interleukin-6 achieved at 3 months among those allocated to canakinumab

Treatment group, on-treatment IL-6 threshold	*N*	Incidence rate (*n*)[a]	HR (95% CI)	*P*-value	HR[adjusted] (95% CI)[b]	*P*-value
MACE						
Placebo	1597	4.91 (282)	1 (ref)		1 (ref)	
Canakinumab, IL-6 top tertile[c]	1098	5.66 (213)	1.17 (0.98–1.39)	0.092	0.99 (0.82–1.19)	0.89
Canakinumab, IL-6 middle tertile	1059	3.88 (150)	0.79 (0.65–0.96)	0.0203	0.73 (0.60–0.90)	0.0029
Canakinumab, IL-6 lowest tertile	1079	3.01 (127)	0.60 (0.49–0.74)	<0.0001	0.65 (0.53–0.81)	<0.0001
P-value for trend across categories				<0.0001		<0.0001
MACE+						
Placebo	1597	5.49 (311)	1 (ref)		1 (ref)	
Canakinumab, IL-6 top tertile[c]	1098	6.00 (224)	1.10 (0.93–1.31)	0.27	0.96 (0.80–1.14)	0.62
Canakinumab, IL-6 middle tertile	1059	4.26 (163)	0.78 (0.64–0.94)	0.0090	0.72 (0.60–0.88)	0.0012
Canakinumab, IL-6 lowest tertile	1079	3.50 (146)	0.63 (0.51–0.76)	<0.0001	0.68 (0.56–0.83)	0.0002
P-value for trend across categories				<0.0001		<0.0001
Cardiovascular mortality						
Placebo	1597	1.66 (103)	1 (ref)		1 (ref)	
Canakinumab, IL-6 top tertile[c]	1098	2.54 (102)	1.55 (1.18–2.04)	0.0016	1.27 (0.96–1.70)	0.10
Canakinumab, IL-6 middle tertile	1059	1.34 (55)	0.81 (0.58–1.13)	0.21	0.76 (0.54–1.06)	0.11
Canakinumab, IL-6 lowest tertile	1079	0.58 (26)	0.35 (0.23–0.53)	<0.0001	0.41 (0.27–0.64)	<0.0001
P-value for trend across categories				<0.0001		<0.0001
Total mortality						
Placebo	1597	2.86 (177)	1 (ref)		1 (ref)	
Canakinumab, IL-6 top tertile[c]	1098	4.50 (181)	1.61 (1.31–1.98)	<0.0001	1.33 (1.07–1.65)	0.0108
Canakinumab, IL-6 middle tertile	1059	2.22 (91)	0.78 (0.60–1.00)	0.0486	0.74 (0.57–0.96)	0.0210
Canakinumab, IL-6 lowest tertile	1079	1.12 (50)	0.39 (0.29–0.53)	<0.0001	0.47 (0.34–0.65)	<0.0001
P-value for trend across categories				<0.0001		<0.0001

[a]Per 100 person-years of exposure.
[b]Covariates included in the adjusted multivariable model include age, gender, smoking status, hypertension, diabetes, body mass index, baseline level of IL-6, and baseline level of LDL cholesterol.
[c]Tertile cutpoints for on-treatment IL-6 levels at 3 months were >2.22 mg/L, >1.25–2.22 mg/L, and ≤1.25 ng/L.
The median percent reduction in IL-6 at 3-months was -34.9.

pre-specified analysis of CANTOS further showed that the magnitude of cardiovascular benefits of canakinumab increased substantially in those who achieved the greatest on-treatment reductions in hsCRP and that this group enjoyed significant reductions in cardiovascular and total mortality.[12] CRP concentrations reflect the acute phase response, a shift in the hepatic programme of protein synthesis implicated in host defenses mediated by IL-6. While hsCRP serves as a convenient and reliable marker of the acute phase response (and perhaps as a clinical tool to monitor canakinumab efficacy), substantial evidence from human genetic studies support a potential causal role in atherothrombosis for IL-6, the signal that elicits the acute phase response.[9,10] IL-1 strongly induces IL-6.[13,14] These considerations suggest that canakinumab's reduction in atherothrombotic events involves inhibition of IL-6. The present study offers insight into the operation of this mechanism in a large scale human trial.

In this substudy of 4833 CANTOS participants, as anticipated,[2–4] baseline IL-6 levels associated with increased risk of future cardiovascular events. More importantly, these data suggest for the first time that modulation of the IL-6 signalling pathway is associated with reduced rates of incident cardiovascular events, cardiovascular death, and all-cause mortality, at least with canakinumab. The differential outcomes observed in CANTOS on the basis of achieved IL-6 concentration were robust to the choice of on-treatment thresholds, were minimally affected by adjustment for baseline clinical characteristics including starting IL-6 levels, and were additionally affirmed in a causal inference analysis.

These findings have interest for several reasons. First, although IL-6 likely contributes causally to atherothrombosis[16,17] no prior direct evidence has shown that lowering IL-6 associates with improved cardiovascular outcomes. Rather, inferences with regard to IL-6 signalling and atherothrombosis have come from Mendelian randomization studies,[9,10] from prospective cohorts linking IL-6 levels to future vascular events,[2–4] and from correlational analyses indicating relationships of IL-6 to traditional risk factors, arterial stiffness, subclinical atherosclerosis, and endothelial dysfunction.[18–21]

Second, the current data add to our understanding that 'lower appears to be better' for inflammation, at least following treatment with canakinumab.[22]

Third, a critical issue for the development of anti-inflammatory therapies following CANTOS is whether targeting the pathways that lead to generation of active IL-1β (such as the NLRP3 inflammasome)

or downstream mediators of IL-1 action (such as IL-6 itself) might provide comparable outcome benefits.[23–27] The present data provides strong support in humans for the concept that proportionately greater lowering IL-6 may result in proportionately fewer cardiovascular events.[17] As such, targets that are both upstream and downstream from IL-1β merit careful evaluation not only for benefits but also for risks, as each may present different adverse effect profiles. With specific regard to IL-6, preliminary studies with tocilizumab, a monoclonal antibody targeting IL-6, have shown modest benefits in terms of reduced troponin levels in post myocardial infarction patients, albeit with concomitant elevations of LDL cholesterol.[28] Other IL-6 inhibitors indicated for arthritis such as sirukumab (an IL-6 receptor antagonist) are also under consideration as atheroprotective agents. Whether the benefits of canakinumab and its sequential inhibition of the IL-1 to IL-6 to CRP pathway varies depending on the presence of somatic mutations underlying clonal haematopoiesis requires direct evaluation.[29,30]

Limitations of our study require consideration. As with on-treatment analyses such as those commonly done for LDL cholesterol or blood pressure, the observations made here are no longer formally randomized. The consistency of the findings after multivariable adjustment for a large range of clinical characteristics including baseline IL-6 levels, as well as in the causal inference analysis in which those treated with canakinumab 150 mg were modelled as having counterfactually been treated with placebo provides reassurance in this regard. As previously shown with hsCRP, these findings agree with the hypothesis that the magnitude of the biologic effect of canakinumab on inflammation (and hence on IL-6) exceeds greatly the magnitude of effect associated with other clinical variables that might introduce residual confounding. Finally, while IL-6 levels at baseline and 3 month follow-up were measured among 4833 CANTOS participants, the baseline characteristics of this subgroup are virtually identical to the trial as a whole. While we do not have accurate and systematic measures of IL-6 past 3 months in CANTOS, prior work with IL-6 has shown levels to be substantially stable over long periods of time.[2–6]

To conclude, these analyses of the multinational CANTOS trial provide evidence in humans supporting the hypothesis that modulation of the IL-6 signalling pathway, at least with canakinumab, associates with reduced cardiovascular event rates, independent of lipid lowering. The current data also support evolving concepts that relate defective inflammation resolution to atherosclerosis, and by extension, provide evidence that reversing such effects might in turn reduce clinical event rates.[31]

Supplementary material

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

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