

# Thalidomide treatment and vte biology essay



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The curative usage of thalidomide has focused attending on venous thrombotic events ( VTEs ) .

There appears to be a background rate of 5-10 % VTE in myeloma perchance due to enhanced look of tissue factor and VEGF, acquired cytokine mediated activated protein C opposition and down-regulation of thrombospondin. In intensively treated patients exposed to thalidomide the rate of VTE additions to 10-15 % , the mechanisms taking to this are unsure, but it is known that thalidomide regulates the degree of COX-2, a good described pro-thrombotic factor. Thalidomide may besides modulate the VTE hazard by its effects on cytokine degrees moving on the endothelial cell, a mechanism dependant on the differential apoptotic effects of thalidomide in myeloma plasma cells compared to endothelial cells, which are protected from programmed cell death by lessening of VEGF by thalidomide, In this context, it is known that stressed human umbilical vena endothelial cells ( HUVECs ) up modulate a figure of pro-coagulant factors including PAR-1, P-selectin, E-selectin and tissue factor, with thalidomide protecting these cells from programmed cell death potentially heightening these pro-coagulant effects, there is some clinical grounds for this mechanism in non-myeloma scenes.

The hazard of developing a VTE following thalidomide exposure depends upon a figure of factors including: Disease phase, the type of chemotherapy combination and the supportive therapy used. Patient specific variables besides contribute to the extra hazard of VTE including stationariness, hapless public presentation position, and desiccation. An of import clinical observation is that VTEs occur early after the induction of thalidomide intervention and VTE rates are increased in patients when used in

concurrency with anthracycline and Decadron and can diminish following exposure to bortezomib. The extra hazard of thalidomide associated VTE in myeloma has been managed by a figure of different schemes, ranging from the designation of high hazard patients suited for prophylaxis to contraceptive anticoagulation for all patients.

Aspirin has been suggested to be effectual, but its usage is controversial because of the deficiency of a readily applicable mechanism warranting its usage. The purpose of this analysis is to identify familial fluctuation associated with VTE following thalidomide exposure in myeloma patients, in an attempt to clarify the molecular mechanisms leading to increased hazard.

Materials and methods

## **Clinical samples**

Peripheral blood Deoxyribonucleic acid samples were obtained from 544 myeloma instances derived from three randomised clinical tests comparing standard initiation intervention for showing patients with thalidomide incorporating regimens derived from the MRC Myeloma IX, 1966 patients, the ECOG EA100, 900 patients and the Hovon-50 survey, 400 patients. The dosage of thalidomide ( 100-200mg daily ) was comparable between the three surveys but the chemotherapy combination used differed. The samples were used as the footing for two nested instance control comparings analyzing the familial part to the hazard of VTE as a effect of thalidomide exposure.

In a find set analysis we compared the genotype consequences derived from 157 Myeloma IX patients with VTEs of which 104 were related to thalidomide

exposure and 53 unrelated, to a control group of 315 age and sex matched myeloma patients besides in the test who did non develop a VTE ( 198 thalidomide exposed and 117 non thalidomide exposed ) . To formalize the frequence distributions we carried out a 2nd instance control comparing utilizing 23 patients with VTE treated with thalidomide and 49 thalidomide treated controls. To guarantee homogeneousness of allelomorphic frequences merely patients of European descent were included.

This survey has been approved by The United Kingdom Multicentre Ethics Committee.

## **Clinical tests**

All patients at high hazard of VTE, defined by clinical standards, were identified and contraceptive anti-coagulation was considered by the treating doctor, but it was non specified. The ECOG EA100 survey randomised patients to either Decadron entirely 40mg day-to-day d1-4, d12-15 or the same dosage in combination with thalidomide 200mg daily. In the survey set, from which samples was available ; no thromboprophylaxis was used on either arm. The Hovon-50 survey randomised patients to either: 3 rythms of VAD: Oncovin ( 0. 4 milligram, four rapid extract on yearss 1-4 ) , doxorubicin ( 9 mg/m<sup>2</sup>, four rapid extract on yearss 1-4 ) and Decadron ( 40 milligram orally, yearss 1-4, 9-12, 17-20 ) or the same regimen but with thalidomide replacing the Oncovin ( TAD ) .

Thalidomide was given daily at a dosage of 200 milligram, but could be escalated to 400 mg. All patients in the TAD arm, received thromboprophylaxis with Low Molecular Weight Heparin ( LMWH ) . Incident

instances of VTE were defined utilizing clinical standards, and no showing attack was used. The designation of VTE represents current clinical pattern with initial clinical designation and subsequent verification and definition of the extent of thrombosis utilizing a unequivocal radiological probe. Central venous thrombosis and line related thrombosis were defined by clinical standards and later confirmed by ultrasound.

## **Consequences**

### **Clinical Consequences**

The Myeloma IX analysis is based on 1966 randomized patients, on 984 instances treated with CTD, 557 with CVAD and 425 with MP.

In the intensive tract, the overall rate of VTE was indistinguishable in both weaponries ( Table S1 ) . However, there was a qualitative difference between the two weaponries with DVT being predominant in the thalidomide treated group and line related thrombosis being prevailing in the CVAD group. In the non-intensive tract really few VTEs were seen in the MP group, whereas in the CTD group there were 15.

0 % VTE. The average clip to VTE in each of the groups was about 12 hebdomads from intervention induction. The Hovon-50 survey had VTE rates of 12. 1 % and 11. 8 % in the thalidomide related and standard weaponries severally with average clip to first event of 8. 9 hebdomads.

In the ECOG EA100 study the rates were 17. 0 % and 3. 0 % .

## Genotyping consequences and proof

### SNPlex platform

A pilot survey was ab initio performed utilizing the 96 SNPs genotyped on the SNPlex platform. 138 patients were genotyped from the MRC Myeloma IX test, 46 of these patients had developed a VTE after intervention and were age/sex matched with 92 control patients that did not develop a VTE after intervention. At the time that the pilot was performed it was not possible to stratify patients by intervention arm. Analysis of these SNPs revealed an association between patients who developed a VTE and SNPs within factor V Leiden ( G1691A ) and MTHFR ( C677T ) , in line with antecedently reported surveys.

We did not see any important association with a figure of other familial discrepancies in the curdling cascade. We did find associations in proinflammatory polymorphisms ; TNF $\alpha$  -488 ( aka. -307, LGALS2 intron 1 C3279T, IL1A ( Ser114Ala ) and the IL 10 [ -854 ] booster SNP ( Table 1 and Figure S6 ) . Targeted-tag ArrayThe MRC Myeloma IX survey is the largest of the information sets in this survey and in order to capitalize on this, we chose to concentrate our findings on this survey and to formalize the consequences on combined information sets from Hovon-50 and ECOG EA100 tests. A set of SNPs for proof was defined, by individually finding the distribution of the most important SNPs in the MRC myeloma IX survey, Hovon50 and ECOG EA100 surveys.

Following proving allelomorphic distributions utilizing Fisher ' s test to an empirical P < 0.05, in the final set, 120 SNPs were found to be associated with thalidomide associated VTE, affecting 71 genes ( Table S2 ) . Further <https://assignbuster.com/thalidomide-treatment-and-vte-biology-essay/>

familial theoretical account association analysis in Myeloma IX information is listed in Table S3, allelomorphic and familial theoretical account association for non-thalidomide related VTEs are listed in Table S4 and S5, with allelomorphic and familial theoretical account association for Hovon-50/EA100 tests shown in Tables S6 and S7. With the purpose of placing cistrans modulating the hazard of thalidomide related thrombosis we compared the distribution of the SNPs identified in this analysis between three subgroups, thalidomide associated VTE MRC IX, non thalidomide associated VTE MRC IX and thalidomide associated VTE from the combined Hovon50/ECOG EA100 surveies. A Venn diagram, picturing the overlapping associated cistrans in the three analyses is shown in Figure 2.

### **Validation of thalidomide related VTE associated SNPs**

To formalize the genotyping consequences in the find set, we discarded SNPs with conflicting frequency distributions between surveies.

This attack may hold led to the remotion of a figure of true positives from the analysis because of the little sizes of the Hovon-50 and ECOG EA100 informations sets. SNPs with little consequence sizes were besides removed from the analysis. As a consequence of this procedure we found 24 SNPs ( Table 1 ) associated with VTE in the “ find set ” with consistent distributions in the two proof datasets. Haplotype analysis showed that 6 SNPs were in linkage with a stronger proxy SNP and as such were discarded go forthing 18 validated SNPs associated with thalidomide related venous thrombotic events. A “ Forest secret plan ” with Odds ratios ( ORs ) and Confidence Intervals ( CI ) for the combined and single informations sets of the validated SNPs was generated.

( Figure 3 )

## **Gene-gene interactions**

To analyze gene-gene interactions we looked for pair-wise combinations interceding hazard, the epistatic interactions with a  $P < 0.001$  are shown in Table S10.

## **Recursive partitioning analysis**

In order to develop a prognostic theoretical account for the designation of patients at high hazard of VTE, we foremost divided the combined information set into a preparation and proof set. We so applied the method of recursive breakdown to the preparation set.

In this attack a arrested development tree is built by first happening the SNP which best splits the informations into two groups ( VTE, no VTE ) . This procedure is repeated over and over once more for the single subsets until the subgroups reach a minimal size or no betterment can be made. The 2nd phase in recursive breakdown consists of cross-validation by paring back ( sniping ) the typically complex full tree. The best pruned trees are examined to happen which 1 has the largest categorization rate while utilizing the smallest figure of SNPs.

Sensitivity and specificity are determined for the preparation and proof set. A receiving system operator characteristic ( ROC ) curve is used to find the best sensitiveness and specificity tradeoff. To maximize the size of the information set and therefore to maximize the ability to place relevant SNPs we combined all the information sets into one and indiscriminately divide it into a two 3rd preparation set and one 3rd proof set. The information was <https://assignbuster.com/thalidomide-treatment-and-vte-biology-essay/>



stratified by test and VTE instances to see that the preparation and proof sets are comparable. This information included 165 topics without VTE and 84 topics with VTE in the preparation set and 82 topics without VTE and 42 topics with VTE in the proof set. The preparation set was used to place the top associated cistrons and SNPs by association at the degree of  $P < 0.05$  listed in Table S2.

These SNPs were used in a recursive breakdown analysis carried out on the trial set with the purpose of happening the combination with the best sensitiveness and specificity for the designation of VTE. We pruned the tree to happen the tree with highest categorization and smallest figure of SNPs. The consequences of this analysis ( Figure 4 ) showed that utilizing 7 SNPs ( rs7011 in CINP, rs289747 in CETP, rs610529 in ALDH1A1, rs3829963 in CDKN1A, rs2608555 in GAN, rs699947 in VEGF and rs168351 in ALDH1A1 ) it was possible to place VTE right in 70 % of persons with a specificity of 59 % and sensitiveness of 81 % . This set of SNPs performed good in the proof set being able to correctly sort VTE in 61 % of persons with a specificity of 30 % and sensitiveness of 77 % . ( Tables S12 and S13 )

## **Discussion**

This survey has analysed informations from three big randomised clinical surveies consisting 3100 patients, comparing initiation intervention for freshly showing patients with myeloma with and without thalidomide. The consequences of this analysis show that the background rate of VTE in MP-treated patients is really low and significantly increases with the add-on of thalidomide. In add-on we provide farther grounds that infusional

governments based on VAD addition VTE rates to around 15 % , which is similar to the rates seen with unwritten thalidomide combinations.

The nature of the thrombotic events is qualitatively different between governments, with all events being either DVT or PE in the unwritten thalidomide treated patients, whereas in the endovenous intervention 50 % of the events are cardinal line related. There is a doubling of non cardinal line related VTE rates in the thalidomide treated patients compared to those having infusional initiation regimens. The average clip to VTE in each of the interventions is about 50-60 years after the induction of intervention, a clip reflecting the rapid disintegration of the myeloma ringer. We have shown antecedently that response rate is enhanced in thalidomide incorporating governments compared to VAD like governments and we postulate that this is of import in finding the VTE hazard. The mechanistic importance of increased response rates with VTE hazard, may explicate the decreased Numberss of VTEs seen in backsliding patients, who are often drug resistant and demo lower response rates. It is besides of import non to dismiss increased VTE hazard due to alterations in the disease biological science related pro-coagulant profiles of such relapse- patients.

Using a nested instance control design, with readily defined exposure and clinical end point, this survey has given utile information about inherited familial discrepancies with a moderate consequence size impacting the thrombotic response to thalidomide exposure. We chose to utilize the MRC Myeloma IX survey as our initial find set because it was the largest and had the most informations available with it. Validation in the combined Hovon50/ECOG survey represents a matter-of-fact determination based on <https://assignbuster.com/thalidomide-treatment-and-vte-biology-essay/>

survey size, survey design and our desire to place penetrant discrepancies that can be replicated with relevancy to different surveys and information sets. Despite a comprehensive analysis of the familial fluctuation within the curdling and pro-thrombotic tracts, we could not happen grounds for an important association of familial fluctuation within these tracts with VTE hazard following thalidomide exposure. Although we found Factor 5 Leiden ( rs6025 ) to be associated with an increased hazard of VTE in this analysis, the thrombotic hazard was not increased in patients treated with thalidomide ; similar consequences were seen for polymorphisms in MTHFR and FGB. We saw no association with thalidomide related VTE in normally reported VTE hazard allelomorphs in F2 -455G/A ( rs3136430 ) splicing variant 20210G/A ( rs3136431 ) .

We did happen weak associations with cistrons known to intercede the curdling pathway including MTRR, PLAUR, PPARD, PPARGC1A, PPARGC1B, THBS4 and WNK, but the associated hazard was not high. We conclude that we can expect a major part of familial fluctuation within the curdling and pro thrombotic tracts based on this targeted attack, although smaller part to the phenotype may be missed because of the survey size and design. Our findings are consistent with old clinical observations and work by some of the writers, who failed to place relevant alterations in functional checks looking into this tract. The deficiency of a strong association with fluctuation in the curdling cascade suggests that VTE hazard is mediated via alternate mechanisms. We identified the following SNPs which validated across the three information sets ( Figure 3 ) . Using the whole BOAC panel as the background cistron set in the DAVID Functional Annotation Clustering tool

against the 18 validated cistrons, generated three major enriched note bunchs. The note bunchs consisted of two ' response to emphasize ' groups ; a response to DNA harm group including: CHEK1, XRCC5, LIG1, ERCC6, DCLRE1B and PARP1 ; and a cytokine response group incorporating NFKB1, TNFRSF17, IL12B and LEP ; and a 3rd related group of ' apoptosis ' with CASP3, PPARD and NFKB1.

These enrichment groups indicate that familial fluctuation in response to DNA harm and cytokine mediated programmed cell death modulates hazard of developing a thalidomide related thrombosis. High dosage Decadron enhances hemostasis, increases thrombocyte activation and promotes von Willebrand factor antigen ( vWF ) dependant thrombosis. Highly high degrees of factor VIII-coagulant ( FVIII: C ) activity and vWF have been found in thalidomide exposed patients. Patients that develop a subsequent VTE had higher VWF-Ag degrees but non FVIII: C.

High FVIII: C/VWF-Ag degrees are found in patients with active myeloma and this is likely a contemplation of increased bone marrow angiogenesis in myeloma. These pro-thrombogenic fortunes would lend to VTE during intervention with a thalidomide-dexamethasone combination. In line with vWF interceding the pro-thrombotic effects of Decadron in thalidomide related VTE, we saw a protective consequence of vWF non-synonymous SNP ( rs216321 ) and synonymous SNP ( rs216902 ) in thalidomide treated controls. Although there is grounds to propose thalidomide may damage DNA straight, it is of import to observe the bulk of instances in this analysis were derived from the MRC and Hovon-50 surveies that included either cyclophosphamide or doxorubicin/adriamycin in the intervention

government, which may explicate an association with DNA fix cistrons.

Variation in DNA fix capacity could readily impact the response of the myeloma ringer to intervention, due to the direct relationship between the extent of DNA harm accretion and the clinical response to alkylating agents.

A rapid response and disintegration of myeloma ringers with an impaired two-base hit stranded DNA fix tract would let go of greater pro-thrombotic factors that could be either micro-particles with surface tissue factor or cytokines and tissue factor. The greater thrombogenesis due to increased disintegration of the myeloma ringer may move additively with a dexamethasone-thalidomide interaction on plasma cells, giving rise to an increased figure of VTEs in the MRC and HOVON surveies. An alternate mechanism to explicate the addition hazard of a VTE associated with DNA fix cistrons could be based on the observation that thalidomide can protect endothelial cells from doxorubicin-induced programmed cell death by reconstructing PAR-1 look advancing sub-endothelial tissues factor exposure, endothelial dysfunction, thrombocyte activation and accordingly increase the thrombosis hazard. Under these conditions decreased DNA fix capacity could advance coagulum formation at the endothelium. The enrichment of cytokine mediated programmed cell death cistrons in SNPs associated with thalidomide related thrombosis hazard may besides give hints to the function bortezomib and aspirin drama in VTE direction. Low rates of VTE are seen in myeloma patients treated with bortezomib ( Velcadea,,? ) in thalidomide combinations, perchance through the bar of the up ordinance of pro-thrombotic molecules such as thrombomodulin, cytokines, and E-selectin by bortezomib. A figure of clinical surveies have suggested that

acetylsalicylic acid is effectual at forestalling the surplus of VTE seen in thalidomide exposed persons.

Aspirin is classically thought to suppress platelet COX-2, cut downing thrombocyte adhesion, modulating hazard of arterial thrombosis. Aspirin can besides take to decrease degrees of go arounding TNF- $I\pm$  , by suppressing IKK and hence NF $\kappa$ B. Higher degrees of TNF- $I\pm$  and COX-2 lead to an increased hazard of programmed cell death in endothelial cells, which besides become pro-adhesive to non-activated thrombocytes. In a thalidomide intervention scene, acetylsalicylic acid may be able to suppress thalidomide VTE mediated events by take downing go arounding TNF- $I\pm$  . Familial analysis of the multi-factorial phenotype that is thalidomide related venous thrombosis is disputing. To understatement experimental artifacts that can be found in many association surveies, we have associated a distinct clinical result from a homogeneous population of likewise treated patients with high quality genotype informations with rigorous quality controls.

We took a hypothesis driven candidate cistron attack instead than a whole genome scan ( WGS ) based attack, because it was clear that the figure of events to be analysed would be little and we were taking to place pertinent functional venue discrepancies with moderate to big consequence size. We accept that future GWS and sequencing attacks may add relevant discrepancies in unknown tracts. As portion of the analysis we took an explorative attack to specifying whether the SNPs identified could be used to place patients at high hazard of VTE and accordingly steer clinical intercession. Guidelines have late been established to regulate clinical indexes for intercession but these predictive factors can be hard to place and

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utilize clinically. The US Food and Drug Administration ( FDA ) and EMEA have published warnings proposing the usage of thromboprophylaxis with any IMiD based regimen. The consequences of this recursive breakdown analysis have identified a limited figure of SNPs which when analysed together can foretell the hazard of VTE.

Testing for these SNPs has the potency for being clinically utile for placing high hazard patients for whom curative intercession is required. For clinically defined high hazard patients intercession schemes may non alter, but for patients at familial high hazard for whom acetylsalicylic acid was the chosen scheme, intercession with Coumadin or LMWH would be more appropriate.