Kawasaki disease (KD) is an acute systemic vasculitis that predominantly affects children, and can result in coronary artery lesions (CAL). A patient with KD who is resistant to treatment with intravenous immunoglobulin (IVIG) has a higher risk of developing CAL. Incomplete KD has increased in prevalence in recent years, and is another risk factor for the development of CAL. Although the pathogenesis of KD remains unclear, there has been increasing evidence for the role of genetic susceptibility to the disease since it was discovered in 1967. We retrospectively reviewed previous genetic research for known susceptibility genes in the pathogenesis of KD, IVIG resistance, and the development of CAL. This review revealed numerous potential susceptibility genes including genetic polymorphisms of ITPKC, CASP3, the transforming growth factor-β signaling pathway, B lymphoid tyrosine kinase, FCGR2A, KCNN2, and other genes, an imbalance of Th17/Treg, and a range of suggested future treatment options. The results of genetic research may improve our understanding of the pathogenesis of KD, and aid in the discovery of new treatment modalities for high-risk patients with KD.

Key words: Kawasaki disease, Genetic susceptibility, Polymorphism, Coronary artery lesion

Introduction

Kawasaki disease (KD) is a multisystemic vasculitis that predominantly affects children, and is associated with coronary artery involvement. The annual incidence of KD is increasing; a nationwide Korean survey of the disease reported an incidence of 115.4/100,000 children under 5 years of age in 2009, which rose to 133.4/100,000 children under 5 years of age in 2011. The incidence of KD in Korea is second only to Japan, where 206.2/100,000 children in 2009, and 239.6/100,000 children in 2010, were affected. The incidence of incomplete KD, which accounts for between 15% and 47% of all KD cases, is also reported to be increasing, posing a threat to the health of coronary arteries in children.

The introduction of intravenous immunoglobulin (IVIG) decreased the incidence of coronary artery lesions (CAL) to less than 5%. However, approximately 10%–20% of patients with KD are reported to be unresponsive to IVIG treatment, and remain at high risk for developing CAL.

Despite the passing of almost 50 years since this complex disease was first recognized, the pathogenesis of KD remains unclear. It is generally accepted that certain infectious agents may trigger KD in genetically predisposed patients. However, although the peak age (6–11 months), seasonality, and symptoms of the disease mimic those of an infectious disease, no infectious agent has been established as a trigger. In addition to a predilection for Asian populations, the fact that the incidence in siblings of KD patients, and in offspring of parents with a history of KD, is 6–10 times higher, and twice that of the general
population, respectively, indicates that genetic susceptibility may have an important role in the pathogenesis of this disease.\textsuperscript{5,10} It is considered very important to predict which patients are resistant to IVIG treatment, and therefore are at an increased risk of developing CAL.

Although a few susceptibility genes have recently been identified, we cannot apply the results of genetic studies in the clinical setting to aid early diagnosis or screening of patients at high-risk of coronary aneurysms, because of uncertainty regarding the role of specific genes as risk factor for KD. The high cost of genetic testing is also a limiting factor. Herein, a summary of several susceptibility genes associated with KD, IVIG resistance, and CAL, is provided, and possible candidate genes and the direction of future treatment are discussed, based on a review of the literature.

**ITPKC and CASP3**

In 2008, Onouchi et al.\textsuperscript{11} reported that functional single-nucleotide polymorphisms (SNPs) in inositol 1,4,5-trisphosphate 3-kinase C (ITPKC) on chromosome 19q13.2 were significantly associated with KD susceptibility, as well as an increased risk of CAL, in both Japanese and American children. ITPKC acts as a negative regulator of T-cell activation through the Ca\textsuperscript{2+}/nuclear factor of activated T cells (NFAT) signaling pathway, and it is thought that the C allele may contribute to immune hyper-reactivity in KD. The ITPKC polymorphism may result in increased activation of T cells with an increased expression of interleukin (IL) 2. This in turn could result in a greater and more prolonged expansion of proinflammatory T cells during the acute phase, which may lead to KD susceptibility and greater disease severity. In the same study, the ipkc_3 C allele was overexpressed in Japanese subjects with a positive family history of KD (n=101; odds ratio [OR], 2.46; 95% confidence interval [CI], 1.63–3.73), and was associated with an increased risk of developing CAL in Japanese subjects, n=106; OR, 2.05; 95% CI, 1.37–3.08; American subjects, n=108; OR, 3.36; 95% CI, 1.72–4.96).

Onouchi et al.\textsuperscript{12}, who performed a positional candidate gene study of the 4q35 region in 2010, in an attempt to identify a novel susceptibility gene, found that there was a set of multiple variants in the caspase-3 (CASP3) gene, which was significantly associated with KD in both Japanese and European American subjects. They found that a G to A substitution of one commonly associated SNP located in the 5′ untranslated region of CASP3 (rs72689236), abolished binding of NFAT to the DNA sequence surrounding the SNP. However, the SNPs of CASP3 were not significant in patients who developed CAL or responded inadequately to IVIG therapy, in either the Japanese or American populations. This indicates that the CASP3 SNPs influence disease susceptibility, but not disease outcome.

In another study by the same authors\textsuperscript{13}, functional SNPs in both ITPKC (rs28493229) and CASP3 (rs113420705; formerly rs72689236) are associated with an increased risk of unresponsiveness to IVIG therapy. Patients with at least one susceptible allele at both loci had a higher risk for unresponsiveness to IVIG therapy, and the development of CAL. The authors suggested that in the subphenotype of patients at high risk of CAL, who are resistant to 2 courses of IVIG and have a prolonged fever of up to 10 days duration, the high-risk trait is determined in part by genetic components.

A later study confirmed the association of the ITPKC rs28493229 SNP with KD in a meta-analysis of 3 case-control studies, undertaken in the Taiwanese population\textsuperscript{14}. The same group also reported a marginal association of the CASP3 rs113420705 A allele with KD\textsuperscript{15}. It is likely that variations of ITPKC and CASP3 confer susceptibility to KD and the development of CAL in different ethnic groups\textsuperscript{14}.

**Th17/Treg imbalance**

Jia et al.\textsuperscript{16}, demonstrated that the proportion of T helper type 17 (Th17) cells, and expression levels of cytokines (IL-17, IL-6, and IL-23) and transcription factors (IL-17A/F, retinoic acid-related orphan receptor) were significantly up-regulated in children with acute KD. Conversely, the proportion of regulatory T cells (Treg) and the expression level of Treg transcription factor (forkhead box P3 [FoxP3]) were significantly down-regulated in the same population. Treg cells expressing FoxP3 have an anti-inflammatory role and maintain tolerance to self-components by contact-dependent suppression, or the release of anti-inflammatory cytokines, such as IL-10 and transforming growth factor (TGF)-β1. IL-17A/F is produced by Th17 cells and has proinflammatory properties, acting on a broad range of cell types to induce the expression of cytokines, including IL-6, tumor necrosis factor alpha, IL-8, and granulocyte-macrophage colony-stimulating factor; IL-17A/F therefore perpetuates inflammation of the tissues. In this study, the proportion of Th17 cells was significantly up-regulated during the acute phase in patients with IVIG-resistant KD, compared with the IVIG-sensitive group. The plasma IL-17A, IL-6, and IL-23 concentrations were significantly higher, while the plasma TGF–β concentration was markedly lower in patients with KD, compared with levels in normal controls and infectious disease patients. This suggests that a Th17/Treg cell imbalance exists in patients with KD, and may be an important factor causing disturbed immunological function, and resulting in IVIG-resistant KD.
TGF-β signaling pathway

Shimizu et al. reported that in 771 KD patients of mainly European descent, the TGF-β pathway influenced KD susceptibility, disease outcome, and response to therapy. In the cardiovascular system, TGF-β can induce neoangiogenesis, cardiomyocyte hypertrophy, calcification, and fibrosis. The TGF-β pathway is also important in inflammation and tissue remodeling mediated by endothelial cells, fibroblasts, and smooth muscle cells. In the immune system, TGF-β modulates the balance of proinflammatory/anti-inflammatory T cells, through a complex set of interactions. It is therefore plausible that genetic variations in the TGF-β pathway may lead to an imbalance of proinflammatory and regulatory T cells by affecting Foxp3 expression, mediated through Smad3 and NFAT. In this study, genetic variations in TGFβ2, TGFβ2, and SMAD3 and their haplotypes were consistently and reproducibly associated with KD susceptibility, coronary artery aneurysm formation, aortic root dilatation, and IVIG treatment response, in different cohorts. One SNP in TGFβ2 (rs2796817), 1 SNP in TGFβ2 (rs11466480), and 5 SNPs in SMAD3 were associated with KD susceptibility. A SMAD3 haplotype associated with KD susceptibility replicated in 2 independent cohorts, and an intronic SNP in a separate haplotype block, were also strongly associated (A/G, rs4776338) (P=0.000022; OR, 1.50; 95% CI, 1.25–1.81). Three SNPs in TGFβ2 (rs10482751, rs2027567, rs12029576), and 2 SNPs in SMAD3 (rs12910697, rs4776339) were consistently associated with CAL in this study. Twenty SNPs in 8 genes in the pathway, including TGFβ2, TGFβ2, and SMAD3, were significantly associated with the maximal internal diameter of the aortic root, normalized for body surface area. One SNP (rs9310940) within TGFβ2 and 1 SNP (rs12901071) within SMAD3 were significant in both the analysis of coronary outcome, and the analysis of aortic root dilatation.

In our previous study, 1 SNP (rs6550004) of TGFβ2 gene was associated with the development of KD. One SNP (rs1495592), formerly classified as of the TGFβ2 gene, was associated with the development of CAL in patients with KD; it was subsequently reclassified as having an unknown function. Another report from our group revealed that 1 SNP (rs3206634; T>C) of SMAD5 was associated with susceptibility to KD, but not associated with patients with CAL in a recessive model. This suggests that the minor allele C may increase the risk of development of KD approximately 2.3 folds, compared with the control group in the Korean population.

B lymphoid tyrosine kinase

B lymphoid tyrosine kinase (BLK) and CD40 SNPs have been implicated in patients with KD in 2 recent genome-wide association studies (GWAS) conducted in Taiwanese and Japanese populations. In 2013, the International Kawasaki Disease Genetics Consortium performed a replication study, which confirmed that the BLK gene was significantly associated with KD in populations of Korean and European descent.

BLK is an Src family tyrosine kinase expressed primarily in the B cell lineage, which transduces signals downstream following stimulation of B cell receptors. The BLK expression pattern during the acute and convalescent stages of KD correlated with the percentage of B cells in the peripheral blood mononuclear cells. The expression of BLK in leukocytes was significantly induced during the acute stage in KD patients, compared with age-matched febrile controls; the expression level decreased after IVIG treatment, and was further reduced at the convalescent stage. It has been shown that the risk allele rs2736340 is associated with a lower expression of BLK in peripheral blood B cells during the acute stage of KD. It is plausible that this decreased expression may alter B cell function and predispose individuals to KD. These results strongly indicate the possible involvement of B cells in immune homeostasis and the development of KD.

FCGR2A

In a GWAS study and replication analysis undertaken in 2,173 individuals with KD and 9,383 controls, from 5 independent sample collections, 2 loci exceeded the formal threshold for genome-wide significance. Functional polymorphism in the IgG receptor gene FCGR2A (encoding an H131R substitution; rs1801274), within the A allele (coding for histidine), conferred an elevated KD risk. The second locus was at 19q13 (rs2233152 and rs28493229), which confirms previous findings by Onouchi et al. The involvement of the FCGR2A locus may have implications for understanding immune activation in KD pathogenesis and the mechanism of response to IVIG.

KCNN2

Kim et al. reported an SNP (rs17136627) in the calcium-activated potassium channel, subfamily N, member 2 (KCNN2) gene on chromosome 5q22.3 to be associated with a 12.6-fold increase in the risk of CAL (≥5 mm), based on GWAS data from 123 patients without CAL, and 17 patients with CAL. In this study, authors selected KD patients with a medium or giant coronary aneurysm who showed more severe phenotypes; this offered a greater statistical significance for detecting CAL risk loci, despite the small sample size. Although the KCNN2 gene is highly expressed in atrial myocytes and is known to be associated with...
with cardiac disease such as arrhythmias, its biological function in the formation of CAL remains to be elucidated.

Other genes

There have been many reports regarding KD-associated genes from GWAS studies and candidate gene studies, including genes described above. Other KD-associated genes shown to have statistical significance in GWAS studies include CD40, HLA-DQB2, HLA-DOB, NFKBIL1, LTA, NAALADL2, ZFHX3, DAB1, PEL1, COPB2, ERAP1, IGHV, and ABC4, as well as ITPKC, CASP3, BLK, and FCGR2A. In a candidate gene studies, many more genes were shown to be related to KD, CAL, or IVIG resistance (CD40, CD209, RETN, FCGR3B, NOD1, NLRP1, ITPKC, TGFB2, ABO, PEL1, SMAD3, TGFB2, CASP3, ANGPT1, VEGFA, MICB, MICA, BAG6, MSH5, VWA7, FCGR2B, IL10, CCL5, TNFRSF1A, CTLA4, MMP3, MMP12, FGB, CCL3L1, CCR5, PRRC2A, ABHD16A, ITPR3, COL11A2, MBL2, MMP11, MIF, IL1B, BTLN2, TPH2, PDCD1, IL18, HLA-E, TIMP4, HLA-G, CRP, TGF, MMP13, HLA-B, HLA-C, CCR3, CCR2, TIMP2, ACE, PLA2G7, IL1RN, IL4, KDR, CD40LG, AGTR1, CD40, SLC11A1, CRP, TNF, MMP3, HLA-A, HLA-F, NFKBIL1, LTA, MTHFR, HP). They expressed as GeneGo Pathway Maps which is an effort of finding functional association among these genes, that are interrelated forming a large network.

Impact on future treatment

Several scoring systems to predict IVIG treatment non-responsiveness have been devised. Additional genetic features, such as the itpck_3 C allele which was associated with an increased risk of IVIG resistance in an American cohort, may facilitate the use of early and aggressive therapy to prevent CAL. Cyclosporin A (CsA) mediates immunosuppression by blocking calcineurin, an important downstream molecule in the Ca²⁺/NFAT signaling pathway. If further studies confirm the importance of the Ca²⁺/NFAT pathway in T-cell activation in acute KD, then a clinical trial of CsA in IVIG-resistant individuals may be warranted.

It may be that the induction of CASP3 acts as a negative feedback mechanism to regulate activation of the Ca²⁺/NFAT pathway. Since many inhibitors of this pathway, such as CsA and tacrolimus, are in clinical use, further elucidation of the role of CASP3 in the pathophysiology of KD may lead to new preventive and therapeutic strategies for this disease.

Decreased BLK expression in peripheral blood B cells may alter B-cell function and predispose individuals to KD. Results of studies outlined above suggest a role for B cells during acute KD. Understanding the functional implications may facilitate the development of B cell-mediated therapy for KD.

Other than calcineurin inhibitors, the TGF-β signaling pathway also has potential for pharmacological intervention, using drugs such as angiotensin receptor blockers.

 Genetic studies are ongoing, by the efforts of the Korean Kawasaki Disease Genetics Consortium in Korea, as well as the International Kawasaki Disease Genetics Consortium. The use of next generation sequencing, first launched in 2005, allows the inexpensive production of large volumes of sequence data. Short sequences can be generated by immobilizing millions of amplified DNA fragments onto a solid surface, and then performing the sequencing reaction. This has enabled us to understand how genetic differences influence diseases, including KD.

It may be not too long before the use of pharmacogenetic genotyping in patients with KD at high risk of CAL or IVIG resistance, is an option. Personalized medicine may be useful in the prevention of the development of CAL in KD patients, similar to its use in age-related macular degeneration, Parkinson and Alzheimer disease, warfarin resistance in patients with cardiovascular disease, and some kinds of cancer.

Conclusions

We retrospectively reviewed the literature relating to susceptible genes for KD and CAL. It is expected that new therapeutic modalities may be developed for use in patients at increased risk of CAL in the future. Such modalities are dependent upon a better understanding of the pathogenesis of, and genetic susceptibility to, KD.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

References

1. Kim GB, Han JW, Park YW, Song MS, Hong YM, Cha SH, et al. Epidemiologic features of Kawasaki disease in South Korea: data from nationwide survey, 2009–2011. Pediatr Infect Dis J 2014; 33:24–7.
2. Nakamura Y, Yashiro M, Uehara R, Sadakane A, Tsuboi S, Aoyama Y, et al. Epidemiologic features of Kawasaki disease in Japan: results of the 2009–2010 nationwide survey. J Epidemiol 2012;22:216–21.
3. Sonobe T, Kiyosawa N, Tsujiya K, Aso S, Imada Y, Imai Y, et al. Prevalence of coronary artery abnormality in incomplete Kawasaki disease. Pediatr Int 2007;49:421–6.
4. Manlhiot C, Christie E, McCrindle BW, Rosenberg H, Chahal N, et al. Prevalence of coronary artery abnormality in incomplete Kawasaki disease. Pediatr Int 2007;49:421–6.
Yeung RS. Complete and incomplete Kawasaki disease: two sides of the same coin. Eur J Pediatr 2012;171:657-62.
5. Sudo D, Monobe Y, Yashiro M, Mienco MN, Uchera R, Tsuchiya K, et al. Coronary artery lesions of incomplete Kawasaki disease: a nationwide survey in Japan. Eur J Pediatr 2012;171:651-6.
6. Uchera R, Belay ED, Maddox RA, Holman RC, Nakamura Y, Yashiro M, et al. Analysis of potential risk factors associated with nonresponse to initial intravenous immunoglobulin treatment among Kawasaki disease patients in Japan. Pediatr Infect Dis J 2008;27:55-60.
7. Burns JC. Commentary: translation of Dr. Tomisaku Kawasaki's original report of fifty patients in 1967. Pediatr Infect Dis J 2002;21:993-5.
8. Nakamura Y, Yashiro M, Uchera R, Sadakane A, Chihara I, Aoyama Y, et al. Epidemiologic features of Kawasaki disease in Japan: results of the 2007-2008 nationwide survey. J Epidemiol 2010;20:302-7.
9. Uchera R, Yashiro M, Nakamura Y, Yanagawa H. Parents with a history of Kawasaki disease whose child also had the same disease. Pediatr Int 2011;53:511-4.
10. Uchera R, Belay ED. Epidemiology of Kawasaki disease in Asia, Europe, and the United States. J Epidemiol 2012;22:79-85.
11. Onouchi Y, Gunji T, Burns JC, Shimizu C, Newburger JW, Yashiro M, et al. ITPKC functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. Nat Genet 2008;40:35-42.
12. Onouchi Y, Ozaki K, Buns JC, Shimizu C, Hamada H, Honda T, et al. Common variants in CASP3 confer susceptibility to Kawasaki disease. Hum Mol Genet 2010;19:2898-906.
13. Onouchi Y, Suzuki Y, Suzuki H, Terai M, Yasukawa K, Hamada H, et al. ITPKC and CASP3 polymorphisms and risks for IVIG unresponsiveness and coronary artery lesion formation in Kawasaki disease. Pharmacogenomics J 2013;13:52-9.
14. Kuo HC, Yang KD, Joo SH, Liang CD, Chen WC, Wang YS, et al. ITPKC single nucleotide polymorphism associated with Kawasaki disease in a Taiwanese population. PLoS One 2011;6:e17370.
15. Kuo HC, Yu HR, Joo SH, Yang KD, Wang YS, Liang CD, et al. CASP3 gene single-nucleotide polymorphism (rs72689236) and Kawasaki disease in Taiwanese children. J Hum Genet 2011;56:161-5.
16. Kuo HC, Hsu YW, Wu CM, Chen SH, Hung KS, Chang WP, et al. A replication study for association of ITPKC and CASP3 two-locus analysis in IVIG unresponsiveness and coronary artery lesion in Kawasaki disease. PLoS One 2013;8:e69685.
17. Jia S, Li C, Wang G, Yang J, Zu Y. The helper type 17 regulatory T cell imbalance in patients with acute Kawasaki disease. Clin Exp Immunol 2010;162:111-7.
18. Shimizu C, Jain S, Davila S, Hibberd ML, Lin KO, Molkara D, et al. Transforming growth factor-beta signaling pathway in patients with Kawasaki disease. Circ Cardiovasc Genet 2011;4:16-25.
19. Ruiz-Ortega M, Rodriguez-Vita J, Sanchez-Lopez E, Carvajal G, Egido J. TGF-beta signaling in vascular fibrosis. Cardiovasc Res 2007;74:196-206.
20. Clark-GreuelJN, Connolly JM, Sorichillo E, Narula NR, Rapoport HS, Mohler ER 3rd, et al. Transforming growth factor-beta1 mechanisms in aortic valve calcification: increased alkaline phosphatase and related events. Ann Thorac Surg 2007;83:946-53.
21. Tone Y, Furuchichi K, Kojima Y, Tykociński ML, Greene MI, Tone M. Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. Nat Immunol 2008;9:194-202.
22. Choi YM, Shim KS, Yoon KL, Han MY, Cha SH, Kim SK, et al. Transforming growth factor beta receptor II polymorphisms are associated with Kawasaki disease. Korean J Pediatr 2012;55:18-23.
23. Cho JH, Han MY, Cha SH, Jung JH, Yoon KL. Genetic polymorphism of SMAD5 is associated with Kawasaki disease. Pediatr Cardiol 2014;35:601-7.
24. Lee YC, Kuo HC, Chang JS, Chang LY, Huang LM, Chen MR, et al. Two new susceptibility loci for Kawasaki disease identified through genome-wide association analysis. Nat Genet 2012;44:522-5.
25. Onouchi Y, Ozaki K, Burns JC, Shimizu C, Terai M, Hamada H, et al. A genome-wide association study identifies three new risk loci for Kawasaki disease. Nat Genet 2012;44:517-21.
26. Chang CJ, Kuo HC, Chang JS, Lee JK, Tsai FJ, Khor CC, et al. Replication and meta-analysis of GWAS identified susceptibility loci in Kawasaki disease confirm the importance of B lymphoid tyrosine kinase (BLK) in disease susceptibility. PLoS One 2013;8:e72037.
27. Khor CC, Davila S, Brenus WB, Lee YC, Shimizu C, Wright VJ, et al. Genome-wide association study identifies FGFR2A as a susceptibility locus for Kawasaki disease. Nat Genet 2011;43:1241-6.
28. Kim JJ, Park YM, Yoon D, Lee KY, Song MS, Lee HD, et al. Identification of KCN2 as a susceptibility locus for coronary artery aneurysms in Kawasaki disease using genome-wide association analysis. J Hum Genet 2013;58:521-5.
29. Lv YY, Wang J, Sun L, Zhang JM, Cao L, Ding YY, et al. Understanding the pathogenesis of Kawasaki disease by network and pathway analysis. Comput Math Methods Med 2013;2013:893307.
30. Fukunishii M, Kikkawa M, Hamana K, Onodera T, Matsuizaki K, Matsuzomo Y, et al. Prediction of non-responsiveness to intravenous gamma-globulin therapy in patients with Kawasaki disease at onset. J Pediatr 2000;137:172-6.
31. Egami K, Muta H, Ishii M, Suda K, Sugahara Y, Iemura M, et al. Prediction of resistance to intravenous immunoglobulin treatment in patients with Kawasaki disease. J Pediatr 2006;149:237-40.
32. Sano T, Kurotobi S, Matsuizaki K, Yamamoto T, Maki I, Miki K, et al. Prediction of non-responsiveness to standard high-dose gamma-globulin therapy in patients with acute Kawasaki disease before starting initial treatment. Eur J Pediatr 2007;166:131-7.
33. Kobayashi T, Inoue Y, Takeuchi K, Okada Y, Tamura K, Tomomasa T, et al. Prediction of intravenous immunoglobulin unresponsiveness in patients with Kawasaki disease. Circulation 2006;113:2606-12.
34. Metzker ML. Sequencing technologies - the next generation. Nat Rev Genet 2010;11:31-46.
35. Desai AN, Jere A. Next-generation sequencing: ready for the clinics? Clin Genet 2012;81:503-10.
36. Baird PN, Hageman GS, Guymer RH. New era for personalized medicine? Clin Genet 2012;81:503-10.
37. Wurtman RJ. Personalized medicine strategies for managing Parkinson's disease. Clin Exp Ophthalmol 2009;37:814-21.
38. Wurtman RJ, et al. Intrinsic cancer subtypes-next steps into personalized medicine. Crit Rev Clin Lab Sci 2015;52:1-11.
39. Santos C, Sanz-Pamplona R, Nadal E, Graselli J, Perans S, Dienstmann R, et al. Intrinsic cancer subtypes-next steps into personalized medicine. Cell Oncol (Dordr) 2015 Jan 14. [Epub]. http://dx.doi.org/10.1007/s13402-014-0203-7.