LILT
LEGA ITALIANA PER LA LOTTA CONTRO I TUMORI
prevenire è vivere

ALLEGATO B

Bando 2020-21 - Programma 5 per mille anno 2018-2019
Investigator Grant (IG)

TRANSLATIONAL RESEARCH

LILT will support research projects in the field of cancer aimed at improving cancer diagnosis and treatment. Particularly considered will be those translational research projects that promise short-medium term effects in clinical practice, concerning new diagnostic methodologies and new therapies. Multicentric studies with national coordination, aimed at validating new diagnostic methods, diagnostic, prognostic and predictive tumor markers, able to improve the clinical management of cancer patients are potentially eligible for funding. Specific research projects on new oncological therapeutic approaches are also eligible for LILT funding as IG. For this type of grants it is necessary to demonstrate solid preliminary experimental data supported by a rigorous biological rationale.

1. Principal investigator's full name and qualification:
Maurizio Genuardi
(Please include: CV in European format with list of publications; IF end Hi-index)
2. Proposal title: **A founder BRCA1 mutation from Central Italy: a model for population screening for cancer predisposition, including assessment of individual attitudes**

3. Primary area of Relevance **Change promoting**

4. Relevance for the National Health System **Primary and secondary prevention of breast cancer**

5. Institution/University: Dipartimento di Scienze della Vita e Sanità Pubblica, Università Cattolica del Sacro Cuore/Fondazione Policlinico Universitario A. Gemelli IRCCS – Largo Francesco Vito 1, 00168 Rome, Italy; phone 06-30154927; e-mail Maurizio.genuardi@unicatt.it

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9. Place and date Rome 22/01/2021

SELF EVALUATION FORM

1. Investigator's full name: (PI) Maurizio Genuardi .....
2. Total papers.....218... IF... 1213.451 .....
3. Total papers (last 10 years).....72...IF... 377.30 ...
4. Total Papers as first/last author or corresponding author...69.....
5. Total H-index .....42.....

PROPOSAL MAIN BODY

1. Proposal title **A founder BRCA1 mutation from Central Italy: a model for population screening for cancer predisposition, including assessment of individual attitudes**
2. Abstract (1 page)

(Rationale of the study, preliminary results, detailed description of the translational value of the research and the expected impact on the NHS)

The identification of carriers of high risk BRCA gene pathogenic variants (PVs) allows targeted implementation of effective risk reducing strategies. Population screening is currently not an option due to cost effectiveness estimates and limited informativeness of test results,(1). However, studies in Ashkenazi Jews, a population characterized by high frequency of specific founder mutations, showed that this is feasible, acceptable and extremely cost-effective (2). Although a number of founder BRCA mutations have been identified in Italy, screening has not been implemented so far. Herewithin we propose to implement a screening approach for the founder *BRCA1* c.4117G>T mutation in 4 provinces from Central Italy. This variant has been identified as a common founder mutation in high risk families from the Abruzzo and Lazio Regions by the oncogenetic centers of Rome (Genetica Medica, Università Cattolica/Policlinico Gemelli) and L'Aquila (UOSD Assistenza Oncologica Territoriale). Overall, 22 unrelated families harbouring *BRCA1* c.4117G>T have been identified so far, and in 18 of these that were tested for segregation a shared haplotype indicative of a common ancestor (founder effect) was identified. Screening for founder mutations has been found to be feasible and acceptable by cancer patients and their relatives in selected groups (namely, Ashkenazi Jews and Polish populations) (2). Despite the identification of a number of regional founder mutations in Italy, there as yet no data on the feasibility of screening for these common high risk variants and on the attitudes of the population towards this approach. Breast cancer patients ($n \geq 400$) will be prospectively recruited at the time of diagnosis in Oncology, Surgery, and Screening Units from the provinces of L'Aquila, Teramo, Frosinone and Viterbo. Patients will be informed about the project and those who will consent to participate will be administered a family history and a pre-test psychological questionnaire and a blood sample will be drawn for targeted genetic analysis of the founder mutation. Test results will be returned, along with post-test psychological questionnaire, to patients who have consented to be informed about the outcome. This project will allow to implement in a specific population a model of screening for cancer predisposition with the aim to maximize the identification of individuals at high risk of breast/ovarian cancer (HBOC) in order to offer targeted risk reducing measures.

Current selection criteria for HBOC allow to identify only a fraction of individuals at high genetic risk in the general population. Therefore, screening of unselected patients is expected to increase the detection rate of HBOC and the number of at risk individuals who can benefit from targeted primary and secondary prevention thus leading to an overall reduction of the burden of these cancers in the population. While, the project will focus on a specific recurrent mutation in the *BRCA1* gene, it will also serve as a pilot for the potential implementation of wider genetic screening, investigating the whole BRCA genes (and possibly other breast cancer predisposing genes). Importantly, the study will also assess individual attitudes towards genetic screening for hereditary HBOC, with the aim to verify the degree of acceptance and potential psychological consequences of screening in the Italian population. This will fill an important gap, since there are few studies on these aspects in our country, and it is essential to know people's attitudes to devise the best approach for the implementation of oncogenetic screening programs.

3. Introduction

The identification of women at high genetic risk of breast cancer (BC) allows targeted implementation of effective risk reducing strategies. Several genes are involved in hereditary predisposition to BC, the most important being *BRCA1* and *BRCA2*, which, overall account for 6% and 10-20% of BCs and ovarian carcinomas (OC), respectively (3,4). Women who carry pathogenic variants (PV) in the BRCA genes have a cumulative lifetime risk of near 80% for BC and 17-44% for OC development (5). BCs tend to occur at early ages, when the individuals do not undergo regular screening. For these reasons, specific prevention pathways have been devised for BRCA PV carriers: these include 6-monthly breast examination and yearly magnetic resonance imaging (MRI) of the breast; in addition, prophylactic bilateral mastectomy, which reduces breast cancer risk by about 95%, can be considered (NCCN guidelines v 2.2021) (6). Due to limited efficacy of surveillance, bilateral adnexectomy, between 35 and 45 years or upon completion of individual reproductive projects is recommended to reduce OC risk (NCCN guidelines).

4. Background and rationale

The identification of BRCA PV carriers allows doctors to apply personalized primary and secondary prevention protocols. At the same time, it allows carrier women to have knowledge of the risk so that they can be motivated to adhere to intensive screening protocols and make the appropriate choices for their clinical management. High risk genetic variants in BRCA1 and BRCA2 are highly heterogeneous, and differ across families. Therefore, the initial test aimed at making a diagnosis of hereditary BC within a family requires analysis of the whole coding sequence of both genes. Although available technologies have simplified these tests, costs are still relatively high and they are associated with a relatively high rate of results of uncertain significance. In addition, oncogenetic tests are still used less than would be required, due to lack of knowledge by health professionals and scarcity of genetic professionals.

Furthermore, in most cases, the reason for BRCA testing is the presence of personal and family history high risk markers fulfilling eligibility criteria established by national or international guidelines (see for instance the regional decree of Lazio, DCA 189/2017 - Regione Lazio) (7) However, it has been shown that such criteria can exclude from testing up to 50% of VP carriers (8)). For such reasons, in specific populations, such as the Ashkenazi Jews, where the burden of hereditary BC and OC is mostly attributable to three specific founder BRCA VPs, screening of the whole population (regardless of personal/family history of BC/OC) limited to such variants has allowed the identification of a number of healthy carriers who would not have been identified using traditional criteria. These studies have shown that in this population the screening is feasible, well accepted and is not associated with stress increase in the short and long run, and is cost effective (9, 10, 11,12)Overall, little is known about the acceptance of genetic testing for BRCA mutations in Italy (13))

Recently, we have identified a founder PV of the *BRCA1* gene - c.4117G>T - p.(Glu1373*) - which is common in the provinces of Aquila, Teramo and Frosinone; this has been found in over 20 families from these areas. So far, in Italy no data are available for genetic screening programs

based on the identification of founder VPs in the BRCA or other cancer predisposing genes.

5. Experimental design (organized in tasks)

The main specific aim of the study is to identify hereditary breast cancer cases related to a *BRCA1* founder mutation - c.4117G>T - p.(Glu1373*) - in Central Italy (Lazio and Abruzzo) thorugh screening of BC patients

The secondary aims are:

A. To assess the prevalence of the c.4117G>T *BRCA1* founder mutation in unselected cases of breast cancer from Lazio and Abruzzo

B. To assess the clinical characteristics of breast cancer probands with the founder BRCA1 PV

C. To evaluate awareness and acceptability of population-based BRCA testing

Task 1. Patient recruitment (months 1-18 of the project)

Newly diagnosed cases of BC, unselected for age, sex, or family history, will be consecutively recruited among patients ascertained in the provinces of Frosinone, Viterbo, Aquila and Teramo. Patients will enrolled through 1) surgical units, before operation; 2) oncology units (hospital and territorial), at the stage of oncological assessment before or after surgery; 3) breast cancer screening units; 4) tumor boards (through case managers). Patients will be informed by the health professional of the recruiting unit about the study and the implications of the genetic test and will be asked to provide informed consent. A venous blood sample (3 ml) will be obtained from those who consent to the study. For each province, the involvement of clinical centers for recruitment will be coordinated by local LILT sections, who have agreed to participate actively in the project. In the province of L'Aquila (which are in the same province), the coordination of recruitment will be performed by the above mentioned Oncology Units of Aquila and Sulmona. Patients will be asked whether they want to receive test results. For those who agree, these will be conveyed by the enrolling physician upon receipt from the laboratory. Patients with a positive test result will be referred to genetic counseling. Furthermore, referral to genetic counseling centers will also be organized for patients with negative results who request further information on genetic testing or based on the advice of the physician.

Task 2. Genetic analyses (months 3-20 of the project)

Samples will be stored at room temperature for up to 3 days or at -20°C up to 1 month before shipment (at room temperature) to the laboratories. Genetic analyses will be performed in the following laboratories: A) Laboratorio della Sezione di Genetica Medica, Dipartimento di Scienze della Vita e di Sanità Pubblica, Università Cattolica del Sacro Cuore/Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma (for samples collected in the provinces of Frosinone and Viterbo) ; B) Laboratorio di Diagnostica Molecolare Oncologica, DISCAB, Università degli Studi dell'Aquila (for samples collected in the provinces of L'Aquila and Teramo). Samples will be received at the Center Genomic DNA will be extracted from peripheral blood leukocytes, a fragment containing exon 12 of the *BRCA1* gene, where the c.4117G>T variant is located, will be amplified by polymerase chain reaction and then subjected to Sanger sequencing. Samples will be pseudonymized by assigning an alphanumeric code and will be stored at -20°C for 5 years after assessment. For each patient who has agreed to be informed about the test result, a report will be issued and sent to the referring physician.

Task 3: Collection of clinical and pathological information and analysis of associations (months 1-24)

Electronic medical records will be reviewed to extract data on clinical characteristics, including age and clinical stage at diagnosis;age at parity; number of full-term pregnancies; use of oral contraceptive pills and hormone replacement therapy; menopause status; ER, PR, and HER-2/*neu* status, nuclear grade, and clinical stage. Family history will be recorded for 1st and 2nd degree relatives, by asking patients to fill a specific questionnaire at the time of enrollment. These data will be collected for all patients who agree to participate in the project, and results will be

compared between cases positive and negative for the BRCA founder mutation, respectively, using appropriate statistical analyses, as detailed in Section 6 (Further details).

Task 4: Assessment of psychological attitudes (months 1-24)
Changes in distress from pre- to post-testing experienced by carriers and noncarriers will be compared. To address this goal, the first aim is to assess the role of emotional intelligence (Self-Perception; Interpersonal; Decision Making; Self-Expression; Flexibility; Mood) in the decision making skills. To evaluate Emotional Intelligence, the Emotional Quotient Inventory (EQI) (14) will be administered, while for the decision making skills we will use the General Decision Making Style (GMDS) (15). State anxiety will be assessed with the state anxiety subscale of the Spielberger State-Trait Anxiety Inventory (16), Cancer specific distress will be assessed with the Impact of Event Scale (17) specifying the distressing event as cancer, hereditary cancer, risk for cancer, and genetic testing. Baseline data will be collected at the time of recruitment and at the time of return of the test report. Patients will be asked to fill in and hand back specific anonymized questionnaires.

6. Further details on the overall methods that will be used in this project

Patient recruitment. Patients will be approached to participate in the study during an out-patient visit to the hospital or outpatient clinic/breast cancer prevention clinic. They will be informed about the scope of the study and potential outcomes. Those who accept to participate will also be asked to fill in researcher will describe the study to the patient and perform a pre-test counseling about hereditary breast and ovarian cancer, the inheritance of BRCA1/2 susceptibility genes, the process of genetic testing (limited to the founder mutation), cancer risks associated with BRCA1/2 mutations and the potential benefits, limitations, and risks of genetic testing. Specific issues that will be discussed will be the ways that knowledge of BRCA1 mutation status might influence medical management (e.g., oophorectomy, enhanced screening) for themselves and their family members, Possible test result outcomes (e.g., positive or negative) will be also reviewed. After providing written informed consent, the patient will be interviewed in person for details about her medical and lifestyle history and her family history of cancer. Based on data from Tumor Registries, the number of incident cases per year in the 4 provinces involved in the study can be estimated between 800 e 900. We expect to recruit approximately 400 cases in 18 months.
Statistical analyses. For analyses of clinical and pathological correlations, Wilcoxon rank sum test will be used to compare the number of full-term pregnancies and the median age at diagnosis, menarche, and first full-term pregnancy across the two patient groups (*BRCA1*-negative vs *BRCA1* PV carriers). A *P* value less than .05 will be considered significant. The same test will be used to compare median age at diagnosis, median age at menarche, median age at parity, and number of full-term pregnancies with receptor status in the *BRCA1* mutation group. Fisher's exact test will be used to assess the association between type of receptor (ER, PR, or HER-2/*neu*), fluorescent in situ hybridization for HER-2/*neu*, nuclear grade, and clinical stage, use of birth control and use of hormone replacement therapy. A *P* value less than .05 will be considered statistically significant.
For psychological assessment, the standardized mean gain effect size (d) will be used to examine changes in emotional distress from before testing to the time of provision of results, with positive effect sizes indicating increased distress.

7. Work carried out and preliminary results

Molecular testing of high risk breast/ovarian cancer families performed in the diagnostic preventative setting has led to the identification of more than 20 unrelated families segregating the same pathogenic variant of the *BRCA1* gene, c.4117G>T, which causes the introduction of a premature stop codon p.(Glu1373Ter). The families were ascertained in the cancer genetic centers of L'Aquila (University of Aquila; UOSD Assistenza Oncologica Territoriale) and Rome (Policlinico Gemelli). All of them were originary from a territory along the Liri river between Tagliacozzo (L'Aquila, Abruzzo) and Sora (Frosinone, Lazio). The c.4117G>T mutant was found

associated in 40 individuals from 18 families to the A allele of the benign BRCA1 variant c.3119G>A - p.(Ser1040Asn). The analysis with microsatellite markers of the 17q21 region confirmed the presence of a common haplotype of approximately 400 kb. These findings allowed to define *BRCA1* c.4117G> T as a founder mutation, common in the territory of Central Italy between Abruzzo (L'Aquila and Teramo) and Southern Lazio (Frosinone) regions (manuscript in preparation).

8. Expected results and relevant corresponding milestones

With this project we expect to:
-establish the proportion of BC cases that are attributable to a *BRCA1* founder mutation in populations from provinces of Central Italy where this variant has been found to be enriched based on previous testing in cancer family clinics. This will be the first genetic screening trial for breast cancer predisposition in the Italian population. It will represent a pilot both for further screening efforts for different founder BRCA PVs common in other regions of Italy and for the extension of screening to the whole coding sequence of the BRCA genes and possibly of other BC predisposing genes. Importantly, the identification of BC patients carrying the *BRCA1* founder PV will be relevant not only for the proband but also for consanguineous relatives, since it will allow to identify subjects at high risk who are candidates for targeted risk reduction approaches.
Relevant milestones: *First 100 patients enrolled: month 6. First 100 genetic tests performed: month 8. First 250 patients enrolled: Month 12. Interim assessment of the frequency of the BRCA1 founder mutation on at least 200 cases: Month 12.*
-We also expect to verify whether the c.4117G>T founder mutation is associated with specific clinical characteristics. The results of clinical correlation analyses can be helpful to define appropriate management for the probands and their relatives (ie to define the more appropriate risk reducing approaches).
Relevant milestones: *Interim assessment of associations on the first subset of at least 200 cases: month 12.*
-Finally, we expect to verify the degree of acceptance of BRCA genetic screening in an unselected population affected with BC. This will have relevance for the design and implementation of wider genetic screening approaches for cancer predisposition, since no information is available on the population attitude in our country.
Relevant milestones: *Return of pre-test and post-test psychological questionnaires by a subset of 100 patients: month 10. Interim assessment of questionnaires: month 12.*

9. References and relevant publications by the research group, already available

References

1) US Preventive Services Task Force Risk Assessment, Genetic Counseling, and Genetic Testing for BRCA-Related Cancer US Preventive Services Task Force Recommendation Statement JAMA. 2019;322(7):652-665. doi:10.1001/jama.2019.10987

2) R Manchanda, L Sun, S Patel 1, O Evans, J Wilschut, A C De Freitas Lopes, F Gaba, A Brentnall, S Du, B Cui, P Coelho De Soarez, Z Husain, J Hopper, Z Sadique, A Mukhopadhyay, L Yang, J Berkhof, R Legood Economic Evaluation of Population-Based BRCA1/BRCA2 Mutation Testing across Multiple Countries and Health Systems
Cancers 2020, 12, 1929; doi:10.3390/cancers12071929

3) Harter P, Hauke J Heitz F, Reuss A, Kommoss S, Marme F, Heimbach A, Prieske K, Richters L, Burges A, et al. Prevalence of deleterious germline variants in risk genes including BRCA1/2 in consecutive ovarian cancer patients (AGO-TR-1).
PLoS ONE **2017**, 12, e0186043.
4) Buys SS, Sandbach, JF, Gammon, A.; Patel, G.; Kidd, J.; Brown, K.L.; Sharma, L.; Saam, J.; Lancaster, J.;

Daly, M.B. A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes.
Cancer **2017**, 123, 1721–1730.

5) K B Kuchenbaecker, JL Hopper, DR Barnes , KA Phillips, TM Mooij, MJ Roos-Blom, S Jervis, FE van Leeuwen, RL Milne, N Andrieu, DE Goldgar, MB Terry, MA Rookus, DF Easton, AC Antoniou, BRCA1 and BRCA2 Cohort Consortium; L McGuffog, D G Evans, D Barrowdale, D Frost, J Adlard, KR Ong, L Izatt, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers
JAMA 2017 Jun 20;317(23):2402-2416. doi: 10.1001/jama.2017.7112.

6) NCCN Guidelines v2.2021
https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf

7) Decreto Regione Lazio
https://www.regione.lazio.it/binary/rl_sanita/tbl_contenuti/DCA_189__2017.pdf

8) Ranjit Manchanda, Faiza Gaba Population Based Testing for Primary Prevention: A Systematic Review
Cancers 2018, 10(11), 424; https://doi.org/10.3390/cancers10110424

9) Gabai-Kapara, E, Lahad, A, Kaufman B, Friedman E, Segev S, Renbaum P, Beeri R, Gal M, Grinshpun-Cohen, J, Djemal, K, et al. Population-based screening for breast and ovarian cancer risk due to BRCA1 and BRCA2.
Proc. Natl. Acad. Sci. USA **2014**, 111, 14205–14210.

10) Manchanda, R, Burnell, M, Gaba, F, Desai, R, Wardle, J, Gessler, S, Side, L, Sanderson, S, Loggenberg, K, Brady, A.F, et al. Randomised trial of population-based BRCA testing in Ashkenazi Jews: Long-term outcomes.
BJOG **2020**, 127, 364–375

11) Manchanda, R.; Legood, R.; Burnell, M.; McGuire, A.; Raikou, M.; Loggenberg, K.; Wardle, J.; Sanderson, S.;Gessler, S.; Side, L.; et al. Cost-e_ectiveness of population screening for BRCA mutations in Ashkenazi jewish women compared with family history-based testing.
J. Natl. Cancer Inst. **2015**, 107, 380.

12) Manchanda, R.; Loggenberg, K.; Sanderson, S.; Burnell, M.; Wardle, J.; Gessler, S.; Side, L.; Balogun, N.;Desai, R.; Kumar, A.; et al. Population testing for cancer predisposing BRCA1/BRCA2 mutations in the Ashkenazi-Jewish community: A randomized controlled trial.
J. Natl. Cancer Inst. **2015**, 107, 379.

13) Di Pietro ML, Zaçe D, Orfino A, Di Raimo FR, Poscia A, de Matteis E, Turchetti D, Godino L, Bertonazzi B, Franiuk M, Bruzzone C, Varesco L, Lucci-Cordisco E, Genuardi M. Intrafamilial communication of hereditary breast and ovarian cancer genetic information in Italian women: towards a personalised approach.
Eur J Hum Genet. 2020 Sep 14. doi: 10.1038/s41431-020-00723-7.

14) Bar-On, R. . The Bar-On Emotional Quotient Inventory (EQ-i): Rationale, description and summary of psychometric properties. In G. Geher (Ed.), Measuring emotional intelligence: Common ground and controversy (p. 115–145). 2004 Nova Science Publishers.

15) Scott, S. G., & Bruce, R. A. (1995). Decision-making style: The development and assessment of a new measure.
Educational and Psychological Measurement, 1995 55(5), 818-831.

16) R. Ferreira, J. Murray Spielberger's State-Trait Anxiety Inventory: Measuring Anxiety with and without an Audience during Performance on a Stabilometer 1983 https://doi.org/10.2466/pms.1983.57.1.15

17) Horowitz MJ, Wilner N, Alvarez W. Impact of Event Scale: A measure of subjective stress. Psychosomatic Medicine 1979;41(3):209–218. [PubMed: 472086]

Relevant publications form the research group

Di Pietro ML, Zaçe D, Orfino A, Di Raimo FR, Poscia A, de Matteis E, Turchetti D, Godino L, Bertonazzi B, Franiuk M, Bruzzone C, Varesco L, Lucci-Cordisco E, Genuardi M. Intrafamilial communication of hereditary breast and ovarian cancer genetic information in Italian women: towards a personalised approach. Eur J Hum Genet. 2020 Sep 14. doi: 10.1038/s41431-020-00723-7. Online ahead of print. PMID: 32929237.

Pastorino R, Basile M, Tognetto A, Di Marco M, Grossi A, Lucci-Cordisco E, Scaldaferri F, De Censi A, Federici A, Villari P, Genuardi M, Ricciardi W, Boccia S. Cost-effectiveness analysis of genetic diagnostic strategies for Lynch syndrome in Italy. PLoS One. 2020;15:e0235038. doi: 10.1371/journal.pone.0235038. eCollection 2020. PMID: 32609729.

Capoluongo E, De Matteis E, Cucinotto I, Ronzino G, Santonocito C, Tornesello A, De Giorgio MR, Lucci Cordisco E, Minucci A, Genuardi M. A new founder BRCA1 haplotype identified in the Puglia region is associated with a specific age-related cancer onset in three unrelated families. Clin Chem Lab Med. 2020 May 12:/j/cclm.ahead-of-print/cclm-2020-0389/cclm-2020-0389.xml. doi: 10.1515/cclm-2020-0389. Online ahead of print. PMID: 32396136.

Capoluongo E, La Verde N, Barberis M, Bella MA, Buttitta F, Carrera P, Colombo N, Cortesi L, Genuardi M, Gion M, Guarneri V, Lorusso D, Marchetti A, Marchetti P, Normanno N, Pasini B, Pensabene M, Pignata S, Radice P, Ricevuto E, Sapino A, Tagliaferri P, Tassone P, Trevisiol C, Truini M, Varesco L, Russo A, Gori S. BRCA1/2 Molecular Assay for Ovarian Cancer Patients: A Survey through Italian Departments of Oncology and Molecular and Genomic Diagnostic Laboratories. Diagnostics 9 Article number 46 (2019).

Tognetto A, Pastorino R, Castorina S, Condorelli DF, DeCensi A, De Vito C, Magnano A, Scaldaferri F, Villari P, Genuardi M, Boccia S. The Current Practice of Lynch Syndrome Diagnosis and Management in Italy: A Qualitative Assessment. Public Health Genomics. 2019 Dec 5:1-19. doi: 10.1159/000504305. PMID: 31805557

Gori S, Barberis M, Bella MA, Buttitta F, Capoluongo E, Carrera P, Colombo N, Cortesi L, Genuardi M, Gion M, Guarneri V, Incorvaia L, La Verde N, Lorusso D, Marchetti A, Marchetti P, Normanno N, Pasini B, Pensabene M, Pignata S, Radice P, Ricevuto E, Sapino A, Tagliaferri P, Tassone P, Trevisiol C, Truini M, Varesco L, Russo A; AIOM-SIGU-SIBIOC-SIAPEC-IAP. Recommendations for the implementation of BRCA testing in ovarian cancer patients and their relatives. Working Group. Crit Rev Oncol Hematol. 2019 May 25;140:67-72. doi: 10.1016/j.critrevonc.2019.05.012. [Epub ahead of print] Review. PMID: 31176273

Pinto C, Bella MA, Capoluongo E, Carrera P, Clemente C, Colombo N, Cortesi L, De Rosa G, Fenizia F, Genuardi M, Gori S, Guarneri V, Marchetti A, Marchetti P, Normanno N, Pasini B, Pignata S, Radice P, Ricevuto E, Russo A, Tagliaferri P, Tassone P, Truini M, Varesco L. Recommendations for the implementation of BRCA testing in the care and treatment pathways of ovarian cancer patients. Future Oncol. 12:2071-2075 (2016). doi: 10.2217/fon-2016-0189.

Peterlongo P, Catucci I, Colombo M, Caleca L, Mucaki E, Bogliolo M, Marin M, Damiola F, Bernard L, Pensotti V, Volorio S, Dall'Olio V, Meindl A, Bartram C, Sutter C, Surowy H, Sornin V, Dondon MG, Eon-Marchais S, Stoppa-Lyonnet D, Andrieu N, Sinilnikova OM; GENESIS, Mitchell G, James PA, Thompson E; kConFab; SWE-BRCA, Marchetti M, Verzeroli C, Tartari C, Capone G, Putignano AL, Genuardi M, Medici V, Marchi I, Federico M, Tognazzo S, Matricardi L, Agata S, Dolcetti R, Della Puppa L, Cini G, Gismondi V, Viassolo V, Perfumo C, Mencarelli MA, Baldassarri M, Peissel B, Roversi G, Silvestri V, Rizzolo P, Spina F, Vivanet C, Tibiletti MG, Caligo MA, Gambino G, Tommasi S, Pilato B, Tondini C, Corna C, Bonanni B, Barile M, Osorio A, Benitez J, Balestrino L, Ottini L, Manoukian S, Pierotti MA, Renieri A, Varesco L, Couch FJ, Wang X, Devilee P, Hilbers FS, van Asperen CJ, Viel A, Montagna M, Cortesi L, Diez O, Balmaña J, Hauke J, Schmutzler RK, Papi L, Pujana MA, Lázaro C, Falanga A, Offit K, Vijai J, Campbell I, Burwinkel B, Kvist A, Ehrencrona H, Mazoyer S, Pizzamiglio S, Verderio P, Surralles J, Rogan PK, Radice P. FANCM c.5791C>T nonsense mutation (rs144567652) induces exon skipping, affects DNA repair activity, and is a familial breast cancer risk factor. Hum Mol Genet. 2015 Jun 30. pii: ddv251. [Epub ahead of print]

Rebbeck TR, Mitra N, Wan F, Sinilnikova OM, Healey S, McGuffog L, Mazoyer S, Chenevix-Trench G, Easton DF, Antoniou AC, Nathanson KL; CIMBA Consortium, Laitman Y, Kushnir A, Paluch-Shimon S, Berger R, Zidan J, Friedman E, Ehrencrona H, Stenmark-Askmalm M, Einbeigi Z, Loman N, Harbst K, Rantala J, Melin B, Huo D, Olopade OI, Seldon J, Ganz PA, Nussbaum RL, Chan SB, Odunsi K, Gayther SA, Domchek SM, Arun BK, Lu KH, Mitchell G, Karlan BY, Walsh C, Lester J, Godwin AK, Pathak H, Ross E, Daly MB, Whittemore AS, John EM, Miron A, Terry MB, Chung WK, Goldgar DE, Buys SS, Janavicius R, Tihomirova L, Tung N, Dorfling CM, van Rensburg EJ, Steele L, Neuhausen SL, Ding YC, Ejlertsen B, Gerdes AM, Hansen Tv, Ramón y Cajal T, Osorio A, Benitez J, Godino J, Tejada MI, Duran M, Weitzel JN, Bobolis KA, Sand SR, Fontaine A, Savarese A, Pasini B, Peissel B, Bonanni B, Zaffaroni D, Vignolo-Lutati F, Scuvera G, Giannini G, Bernard L, Genuardi M, Radice P, Dolcetti R, Manoukian S, Pensotti V, Gismondi V, Yannoukakos D, Fostira F, Garber J, Torres D, Rashid MU, Hamann U, Peock S, Frost D, Platte R, Evans DG, Eeles R, Davidson R, Eccles D, Cole T, Cook J, Brewer C, Hodgson S, Morrison PJ, Walker L, Porteous ME, Kennedy MJ, Izatt L, Adlard J, Donaldson A, Ellis S, Sharma P, Schmutzler RK, Wappenschmidt B, Becker A, Rhiem K, Hahnen E, Engel C, Meindl A, Engert S, Ditsch N, Arnold N, Plendl HJ, Mundhenke C, Niederacher D, Fleisch M, Sutter C, Bartram CR, Dikow N, Wang-Gohrke S, Gadzicki D, Steinemann D, Kast K, Beer M, Varon-Mateeva R, Gehrig A, Weber BH, Stoppa-Lyonnet D, Sinilnikova OM, Mazoyer S, Houdayer C, Belotti M, Gauthier-Villars M, Damiola F, Boutry-Kryza N, Lasset C, Sobol H, Peyrat JP, Muller D, Fricker JP, Collonge-Rame MA, Mortemousque I, Nogues C, Rouleau E, Isaacs C, De Paepe A, Poppe B, Claes K, De Leeneer K, Piedmonte M, Rodriguez G, Wakely K, Boggess J, Blank SV, Basil J, Azodi M, Phillips KA, Caldes T, de la Hoya M, Romero A, Nevanlinna H, Aittomäki K, van der Hout AH, Hogervorst FB, Verhoef S, Collée JM, Seynaeve C, Oosterwijk JC, Gille JJ, Wijnen JT, Garcia EB, Kets CM, Ausems MG, Aalfs CM, Devilee P, Mensenkamp AR, Kwong A, Olah E, Papp J, Diez O, Lazaro C, Darder E, Blanco I, Salinas M, Jakubowska A, Lubinski J, Gronwald J, Jaworska-Bieniek K, Durda K, Sukiennicki G, Huzarski T, Byrski T, Cybulski C, Toloczko-Grabarek A, Złowocka-Perłowska E, Menkiszak J, Arason A, Barkardottir RB, Simard J, Laframboise R, Montagna M, Agata S, Alducci E, Peixoto A, Teixeira MR, Spurdle AB, Lee MH, Park SK, Kim SW, Friebel TM, Couch FJ, Lindor NM, Pankratz VS, Guidugli L, Wang X, Tischkowitz M, Foretova L, Vijai J, Offit K, Robson M, Rau-Murthy R, Kauff N, Fink-Retter A, Singer CF, Rappaport C, Gschwantler-Kaulich D, Pfeiler G, Tea MK, Berger A, Greene MH, Mai PL, Imyanitov EN, Toland AE, Senter L, Bojesen A, Pedersen IS, Skytte AB, Sunde L, Thomassen M, Moeller ST, Kruse TA, Jensen UB, Caligo MA, Aretini P, Teo SH, Selkirk CG, Hulick PJ, Andrulis I. Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. JAMA. 313: 1347-1361 (2015).

Hehir-Kwa JY, Claustres M, Hastings RJ, van Ravenswaaij-Arts C, Christenhusz G, Genuardi M, Melegh B, Cambon-Thomsen A, Patsalis P, Vermeesch J, Cornel MC, Searle B, Palotie A, Capoluongo E, Peterlin B, Estivill X, Robinson PN. Towards a European consensus for reporting incidental findings during clinical NGS testing.Eur J Hum Genet. 2015 Jun 3. doi: 10.1038/ejhg.2015.111. [Epub ahead of print].

Thompson BA, Spurdle AB, Plazzer JP, Greenblatt MS, Akagi K, Al-Mulla F, Bapat B, Bernstein I, Capellá G, den Dunnen JT, du Sart D, Fabre A, Farrell MP, Farrington SM, Frayling IM, Frebourg T, Goldgar DE, Heinen CD, Holinski-Feder E, Kohonen-Corish M, Robinson KL, Leung SY, Martins A, Moller P, Morak M, Nystrom M, Peltomaki P, Pineda M, Qi M, Ramesar R, Rasmussen LJ, Royer-Pokora B, Scott RJ, Sijmons R, Tavtigian

SV, Tops CM, Weber T, Wijnen J, Woods MO, Macrae F, Genuardi M. Application of a five-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants lodged on the InSiGHT locus-specific database. Nat Genet. 46:107-115 (2014).

Gaudet MM, Kuchenbaecker KB, Vijai J, Klein RJ, Kirchhoff T, McGuffog L, Barrowdale D, Dunning AM, Lee A, Dennis J, Healey S, Dicks E, Soucy P, Sinilnikova OM, Pankratz VS, Wang X, Eldridge RC, Tessier DC, Vincent D, Bacot F, Hogervorst FBL, Peock S, Stoppa-Lyonnet D, Peterlongo P, Schmutzler RK, Nathanson KL, Piedmonte M, Singer CF, Thomassen M, v.O. Hansen T, Neuhausen SL, Blanco I, Greene MH, Garber J, Weitzel JN, Andrulis IN Goldgar DE, D'Andrea E, Caldes T, Nevanlinna H, Osorio A, van Rensburg EJ, Arason A, Rennert G, van den Ouweland AMW, van der Hout A, Kets CM, Aalfs CM, Wijnen JT, Ausems MGEM, Frost D, Ellis S, Fineberg E, Platte R, Evans DG, Jacobs C, Adlard J, Tischkowitz M, Porteous ME, Damiola F, Golmard L, Barjhoux L, Longy M, Belotti M, Ferrer SF, Mazoyer S, Spurdle AB, Manoukian S, Barile M, Genuardi M, Arnold N, Meindl A, Sutter C, Wappenschmidt B, Domchek SM, Pfeiler G, Friedman E, Jensen UB, Robson M, Shah S, Lazaro C, Mai PL, Benitez J, Southey MC, Schmidt MK, Fasching PA, Peto J, Humphreys MK, Wang Q, Michailidou K, Sawyer EJ, Burwinkel B, Guénel P, Bojesen SE, Milne RL, Brenner H, Lochmann M, Aittomäki K, Dörk T, Margolin S, Mannermaa A, Lambrechts D, Chang-Claude J, Radice P, Giles GG, Haiman CA, Winqvist R, Devillee P, García-Closas M, Schoof N, Hooning MJ, Cox A, Pharoah PDP, Jakubowska A, Orr N, González-Neira A, Pita G, Alonso MR, Hall P, Couch FJ, Simard J, Altshuler D, Easton DF, Chenevix-Trench G, Antoniou AC, Offit K. Identification of a BRCA2-specific Modifier Locus at 6p24 Related to Breast Cancer Risk' in PLOS Genetics. PLOS Genet 9:e1003173 (2013).

Papi L., Putignano A.L., Congregati C., Zanna I., Sera F., Morrone D., Falchetti M., Rosselli Del Turco M., Ottini L., Palli D., Genuardi M. Founder mutations account for the majority of BRCA1-attributable hereditary breast/ovarian cancer cases in a population from Tuscany, Central Italy. Breast Cancer Res. Treat. 117:497-504. (2009)

Greenblatt M.S., Brody L.C., Foulkes W.D., Genuardi M., Hofstra R.M., Olivier M., Plon S.E., Sijmons R.H., Sinilnikova O., Spurdle A.B., IARC Unclassified Genetic Variants Working Group. Locus-specific databases and recommendations to strengthen their contribution to the classification of variants in cancer susceptibility genes. Hum. Mutat. 29:1273-1281 (2008).

Plon S.E., Eccles D.M., Easton D., Foulkes W.D., Genuardi M., Greenblatt M.S., Hogervorst F.B., Hoogerbrugge N., Spurdle A.B., Tavtigian S.V., IARC Unclassified Genetic Variants Working Group. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum. Mutat. 29:1282-1291 (2008).

Caluseriu O., Di Gregorio C., Lucci-Cordisco E., Santarosa M., Trojan J., Brieger A., Colibazzi T., Benatti P., Pedroni M., Bellacosa A., Neri G., Ponz de Leon M., Viel A., Genuardi M. "A founder *MLH1* mutation in hereditary non polyposis colorectal cancer families from the districts of Modena and Reggio-Emilia in Northern Italy associated with protein elongation and instability". J. Med. Genet. 41:E34 (2004).

Greggi S., Genuardi M., Benedetti-Panici P., Cento R., Scambia G., Neri G., Mancuso S. "Analysis of 138 consecutive ovarian cancer patients: incidence and characteristics of familial cases". Gynecol. Oncol. 39:300-304 (1990).

Mars W.M., Genuardi M., Tsihira H., Anderson D.E., Saunders G.F. "Genomic changes on the short arm of chromosome 1 in breast cancer". Cancer Detect. Prev. 15:145-149 (1991).

Genuardi M., Tsihira H., Anderson D.E., Saunders G.F. "Distal deletion of chromosome 1p in ductal carcinoma of the breast". Am. J. Hum. Genet. 45:73-82 (1989).

PERSONNEL INVOLVED IN THE RESEARCH

Name and date of birth	**Role on Project**	**Fellowship required**	**Effort on project (%)**	**Present position**
Genuardi Maurizio 22/09/1957	coordinator	no	20%	Full Professor Catholic University. Director, Medical Genetics, Fondazione Policlinico Gemelli IRCCS
Lucci Cordisco Emanuela 06/09/1974	Genetic analysis	no	20%	Researcher at Catholic University. Genetic consultant, Fondazione Policlinico Gemelli IRCCS
Roberta Pastorino 9/12/1981	Biostatistician	No	10%	Biostatistician at Fondazione Policlinico Gemelli
Chieffo Daniela Pia Rosaria 23/12/1973	Psychological evaluation	no	10%	Director Psychology Unit Fondazione Policlinico Gemelli
Giuseppe D'Ermo 08/10/1956	Patient enrollment and executive coordination	no	10%	Assistant Professor Sapienza University
Enrico Ricevuto 24/11/1961	Patient enrollment. Study design		20%	Director, UOSD Assistenza Oncologica Territoriale, L'Aquila, and UOSD Oncologia, Sulmona
Silvia Ferella 03/09/1987	Genetic analysis		20%	Lab scientist, Laboratorio di Diagnostica Molecolare Oncologica, DISCAB, Università degli Studi dell'Aquila
Benedetto Addari 08/09/1957	Patient enrollment	no	10%	LILT L'Aquila President
Norberto Venturi 27/09/1951	Patient enrollment	no	10%	LILT Frosinone President

Massimo Gemini 28/04/1946	Patient enrollment	no	10%	LILT Viterbo President
Giuseppina Di Massimo	Patient enrollment	no	10%	LILT Teramo President

DESCRIPTION OF THE WORK FOR EVERY UNIT OF PERSONNEL

1.Genuardi Maurizio: P.I, study coordinator, study design
2.Emanuela Lucci Cordisco: study design, responsible for genetic analyses in Rome laboratory
3.Roberta Pastorino will perform statistical analyses
4.Daniela Pia Rosaria Chieffo: design and assessment of psychological tests
5.Giuseppe D'Ermo: involved in study design and overall coordination of the project
6.Enrico Ricevuto: study design, responsible for organization of patient recruitment and supervision of genetic analyses in the Aquila and Teramo provinces
7.Silvia Ferella: responsible for genetic analyses in L'Aquila laboratory
8.Benedetto Addari: coordination of patient recruitment and logistical support in L'Aquila province
9.Norberto Venturi: coordination of patient recruitment and logistical support in Frosinone province
10.Massimo Gemini: coordination of patient recruitment and logistical support in Viterbo province
11.Giuseppina Di Massimo: coordination of patient recruitment and logistical support in Teramo province

Budget Form /year

1. Research costs € 43.100
2. Instruments
3. Indirect costs
4. **Sub-total € 43.100**
5. Overheads € 15.900
6. Fellowships € 48.000
7. **Total € 106.000**

Justifications

Itemized research costs: supplies for genetic tests: 40.000 € (20 €/sample; half already covered by University funds). Printing and shipment of questionnaires: 1.400 €. Sample

storage and shipment: 1.700 €.
Overheads: 15% of total budget: €15.900
Fellowships: 1 fellowship for technical/administrative coordinator (total € 48.000 - 24.000/year)

EXISTING/PENDING SUPPORT Local university funds cover part of the genetic analyses

SUGGESTED REVIEWERS (MAX 3)

1.Paolo Radice, Istituto Tumori Milano IRCCS. Email: paolo.radice@istitutotumori.mi.it
2.Conxi Lazaro, Hereditary Cancer, IDIBELL, Barcelona, Spain. Email: clazaro@idibell.cat
3.Ian Lubinski, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland. Email: Lubinski@pum.edu.pl

BIOETHICAL REQUIREMENT

1. Human experimentation ……(YES) – please provide clearance from the competent ethical committee as addendum A

2. Animal experimentation …..(NOT) – please include a statement as addendum B specifying which regulations the proposed research meets

Declaration
I shall confirm to the Declaration of Helsinki in its latest version.
I shall also apply the Bioethics Convention of the Council of Europe.
In implementing the proposed research, I shall adhere most strictly to all existing ethical and safety provisions applicable.
Before start of the research, I shall obtain clearance from the competent ethical committee in case of involvement of human subjects in the research and /or in case of other ethical implications.

Date: 22/01/2021 Name of PI Maurizio Genuardi signature

Principal investigator's signature ……

Authorized Administrative Official's signature……

Date 22/01/2021

Si autorizza al trattamento dei dati ai sensi dell'articolo 5 del Regolamento (UE) 2016/679